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Wastewater Management

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June 1, 2010

Ms. Kathy Harder
Central Valley Regional Water Quality Control Board
11020 Sun Center Drive, Suite 200
Rancho Cordova, CA 95760-6114

Subject: Sacramento Regional County Sanitation District Comments on Issue Paper Regarding Aquatic Life and Wildlife Preservation Issues

Dear Ms. Harder:

The Sacramento Regional County Sanitation District (SRCSD) appreciates the opportunity to offer comments on the Central Valley Regional Water Quality Control Board's (Water Board) Issue Paper regarding Aquatic Life and Wildlife Preservation Related Issues (Issue Paper), as prepared by Water Board staff. The Issue Paper raises and discusses numerous issues associated with the renewal of SRCSD's NPDES permit and appears to rely on information contained in documents provided by SRCSD to the Water Board as part of the NPDES permit renewal process and on information based on research studies that are currently in progress.

SRCSD's comments are provided in Attachment A under the same general topic areas as in the Issue Paper including:

- Mixing Zones and Dilution for Aquatic Life Criteria
- Ammonia
- Low Dissolved Oxygen
- Thermal Conditions
- Pyrethroid Pesticides
- Whole Effluent Toxicity

SRCSD appreciates the opportunity to provide comments on this Issue Paper. Please feel free to contact Robert Seyfried of my staff at 916-876-6068 or seyfriedr@sacsewer.com if you have additional questions regarding our comments.

Sincerely,

Stan R. Dean
Director of Policy and Planning

Attachments: Attachment A - SRCSD's Comments

cc: Kenneth Landau, CVRWQCB
Diana Messina, CVRWQCB
James Marshall, CVRWQCB
Mary Snyder, SRCSD
Ruben Robles, SRCSD
Terrie Mitchell, SRCSD
Robert Seyfried, SRCSD
Vyomini Pandya, SRCSD
Tom Grovhoug, Larry Walker Associates
Betsy Elzufon, Larry Walker Associates
Tess Dunham, Somach Simmons & Dunn
Debbie Webster, Central Valley Clean Water Association

Attachment A

Sacramento Regional County Sanitation District (District) Comments on Issue Paper Regarding Aquatic Life and Wildlife Preservation Issues

The Sacramento Regional County Sanitation District ("SRCSD" or the "District") appreciates the opportunity to offer comments on the Central Valley Regional Water Quality Control Board's (Water Board) Issue Paper regarding Aquatic Life and Wildlife Preservation Related Issues (Issue Paper), as prepared by Water Board staff. The Issue Paper raises and discusses numerous issues associated with the renewal of the District's NPDES permit and appears to rely on information contained in documents provided by the District to the Water Board as part of the NPDES permit renewal process and on information based on research studies that are currently in progress.

The District's comments are provided under the same general topic areas as in the Issue Paper:

1. Mixing Zones and Dilution for Aquatic Life Criteria
2. Ammonia
3. Low Dissolved Oxygen
4. Thermal Conditions
5. Pyrethroid Pesticides
6. Whole Effluent Toxicity

MIXING ZONES AND DILUTION FOR AQUATIC LIFE CRITERIA

The Issue Paper raises several questions regarding the proposed mixing zone in the Sacramento River downstream of the Sacramento Regional Wastewater Treatment Plant (SRWTP) discharge. The issue paper references approaches to establishing mixing zones based on the 1995 policy used in EPA Region VIII to guide States and Tribes in that region. Specifically, the Issue Paper refers to the Region VIII document with respect to the applicability of mixing zones to acute aquatic life criteria and with respect to consideration of attraction of aquatic life to the effluent plume. The Issue paper also discusses the applicability of a mixing zone for ammonia based on conditions in the Delta.

While the District's proposed mixing zone meets the criteria proposed by Region VIII, the District urges the Regional Board to rely on the mixing zone policies established under the State Implementation Plan which was adopted by the State Water Resources Control Board in 2000[1] and has been used to establish mixing zones throughout the Central Valley and more generally in the State of California. The District has conducted a thorough effort to model the discharge and evaluate the risks in the near field and, as described in the District's Anti-degradation Analysis[2], there is no unacceptable risk to aquatic life within the District's

[1] California Environmental Protection Agency (Cal EPA). 2000. Policy of Implementation of Toxic Standards for Inland Surface Waters, Enclosed Bays, and Estuaries of California (SIP). State Water Resources Control Board

[2] Larry Walker Associates, 2009. Anti-Degradation Analysis for Proposed Discharge Modification to the Sacramento Regional Wastewater Treatment Plant. DRAFT. Prepared for Sacramento Regional County Sanitation District. May 2009.

proposed mixing zone. As stated below, the proposed mixing zone for the SRCSD discharge meets all applicable State and federal requirements and guidelines and is established in a manner that is consistent with other mixing zones granted by the Central Valley Regional Water Quality Control Board (Central Valley Water Board) in other NPDES permits.

The State's mixing zone policy, as it applies to priority toxic pollutants, is contained in the state's *Policy for Implementation of Toxics Standards for Inland Surface Waters, Enclosed Bays, and Estuaries of California* ("SIP"). The specific SIP mixing zone requirements are that the mixing zone must be as small as practicable and shall not:

- Compromise the integrity of the water body
- Cause acute toxicity conditions to aquatic life passing through the mixing zone;
- Restrict the passage of aquatic life
- Adversely impact biologically sensitive or critical habitats, including, but not limited to, habitats of species listed under federal or State endangered species laws;
- Produce undesirable or nuisance aquatic life;
- Result in floating debris, oil, or scum;
- Produce objectionable color, odor, taste, or turbidity;
- Cause objectionable bottom deposits;
- Cause nuisance;
- Dominate the receiving water body or overlap a mixing zone from different outfall;
- Be located at or near any drinking water intake.

The proposed mixing zone for the Sacramento Regional Wastewater Treatment Plant (SRWTP) discharge satisfies each of these criteria.

The Issue Paper references the guidance for mixing zones that has been developed for USPEA Region VIII. First, it should be noted that California is in USEPA Region IX – not Region VIII. USEPA Region VIII covers the states of Colorado, Utah, Wyoming, North Dakota, South Dakota and Montana. Thus, the USEPA Regional VIII guidance referenced in the issue paper does not apply to California or SRCSD's discharge. The USEPA Region VIII *Mixing Zones and Dilution Policy* is a 1994 document that was developed to upgrade methods for deriving water quality-based permit limits, improve the technical defensibility of NPDES permits, and reduce risks associated with mixing zone and dilution practices in those States within its jurisdiction. The document was specifically developed to address a concern with a 1990's practice in Region VIII states to follow a simple mass balance approach that effectively provided the entire critical low flow as a dilution allowance and granted mixing zones which extended far downstream of a discharge. The guidance states that consideration of how quickly a discharge actually mixes is important in the mixing zone and dilution determination. The purpose of the Region VIII guidance was to implement a mixing zone approach that placed controls on the size and quality of effluent plumes.

On page 10 of the Region VIII guidance document, a definition of "near instantaneous and complete mixing" is provided. This condition is defined as "no more than a 10% difference in bank-to-bank concentrations within a longitudinal distance of not greater than 2 stream/river widths." The provisions of the Region VIII policy vary depending on the determination of whether a discharge is completely or incompletely mixed. For instance, the Region VIII guidance states explicitly that where a discharge mixes rapidly with a receiving water body, a dilution allowance based on the critical low flow of the receiving water may be provided. In cases where a discharge is determined to be incompletely mixed, per the Region VIII definition, the guidance is more restrictive (e.g. consideration should be given to restricting dilution credit for acute limits, etc.).

For incompletely-mixed flows, the Region VIII Guidance Document provides guidance in "Alternative Procedures for Chemical-Specific Acute Criteria in Incompletely-Mixed Situations (Appendix D),"

> *For acute chemical-specific standards in incomplete mix situations, although achieving such standards at the end-of-pipe is recommended by the Region, EPA will also approve mixing zone policies that allow a zone of initial dilution on a case-by-case basis where:*
>
> *There is evidence of rapid mixing between the discharge and receiving water based on factors such as high exit velocity of the discharge (e.g., > 10 ft per second), and*
>
> *The rationale for the discharge permit includes an evaluation of risks (such as those describe in Step 4 of the Region's model procedure) and a finding that allowing a zone of initial dilution posses no unacceptable risk.*
>
> *Where both of the above two conditions are met in a particular case, it is recommended that the zone of initial dilution (ZID) for achieving acute standards be limited as follows:*
>
> *Rivers and Streams: The ZID volume must be small. This may be implemented by applying the more stringent of the following two restrictions:*
>
> *ZID volume or flow may not exceed 10% of the chronic mixing zone volume or flow; or*
>
> *ZID length may not exceed a maximum downstream length of 100 feet.*

Flexibility regarding mixing zones for incompletely mixed discharges is also provided as outlined in the flow chart in Figure 1 of the document. Under Step 5 in Figure 1, a mixing zone and dilution credit may be allowed if there is use of a diffuser which would be applicable to the SRWTP discharge. Under Step 6, dilution may be determined by a field study. The numerous dye studies conducted to validate the District's dynamic model would certainly provide the field validation necessary to document dilution of the SRWTP discharge.

As noted above, the EPA Region VIII Guidance Document was generated to stop the practice of using a "simplified mass balance approach that effectively provides the entire critical low flow as a dilution allowance in calculating the permit limit, regardless of the rate of mixing" (p. 1 of EPA Region VIII Mixing Zones and Dilution Policy). Clearly this is not the situation or the proposal regarding the SRWTP mixing zone. The SRWTP diffuser causes "rapid mixing of effluent into the receiving water within a short distance of the discharge." [3] In addition, the District has conducted a thorough effort to model the discharge and to conduct field studies documenting dilution to evaluate the risks in the near field and, as described in the District's Anti-degradation Analysis[4], there is no unacceptable risk to aquatic life. The edge of the acute mixing zone proposed by the District is 60 feet downstream from the diffuser, which is consistent with the Region VIII guidance of not exceeding 100 feet. Further, the EPA Region VIII Guidance document acknowledges that the document serves as guidance and that States and Tribes should develop their own methods and criteria for setting up acute and chronic mixing zones.

Also, on Page 10, the Region VIII guidance specifies maximum size restrictions on mixing zones, particularly applicable to incompletely mixed discharges. For streams and rivers, mixing zones must not exceed one-half of the cross-sectional area or a length of 10 times the stream width at critical low flow, whichever is more limiting.

In evaluating the proposed SRCSD aquatic life mixing zones (an acute mixing zone extending 60 feet downstream from the diffuser and a chronic mixing zone extending 350 feet downstream) in comparison to the maximum mixing zone size restrictions cited in the Region VIII guidance, neither of those mixing zones would occupy over half of the river cross section or extend more than 6000 feet (10 times the river width) downstream. Therefore, the proposed SRCSD aquatic life mixing zones would satisfy the maximum size provisions of the Region VIII guidance.

The Issue Paper also notes that the Region VIII document recommends meeting acute or chronic water quality criteria without dilution 'where available data support a conclusion that fish or other aquatic life are attracted to the effluent plume.' While the area around the SRWTP outfall is 'known to be popular for fishing,' there is no evidence that this is a result of attraction or that it results in 'adverse effects such as acute or chronic toxicity.' The absence of evidence of fish toxicity supports a finding that adverse effects are not occurring at this location. Lacking evidence or information that adverse effects are occurring near the SRCSD diffuser, special restrictions on the proposed mixing zone are not warranted.

Examples of NPDES permits adopted by the Central Valley Water Board that have been granted acute and chronic mixing zones are shown in Table 1. In addition, acute mixing zones have recently been proposed in the San Francisco Bay Region for the Town of Yountville (Order No. R2-2010-0072) and the City of Calistoga. These NPDES permits in the Central Valley and San Francisco Bay Regions have satisfied the SIP's requirements for mixing zones.

[3] California Regional Water Quality Control Board, Central Valley Regiona. Order No. 5-00-188. NPDES No. CA0077682

[4] Larry Walker Associates, 2009. Anti-Degradation Analysis for Proposed Discharge Modification to the Sacramento Regional Wastewater Treatment Plant. DRAFT. Prepared for Sacramento Regional County Sanitation District. May 2009

TABLE 1 - REGION 5 ADOPTED MIXING ZONES[5]

Discharger	Order #	Type	Receiving Water
City of Chico, Chico Water Pollution Control Plant	R5-2010-0019	Acute, Chronic and Human Health	Sacramento River M&T Irrigation Canal
City of Yuba City, City of Yuba City Wastewater Treatment Facility	R5-2007-0134-01 (as amended by Order No. R5-2010-0007)	Acute, Chronic and Human Health	Feather River
City of Angels, City of Angels Wastewater Treatment Plant	R5-2007-0031-01 (as amended by Order No. R5-2009-0074)	Acute, Chronic and Human Health	Angels Creek
Forest Meadows Wastewater Reclamation Plant, Calaveras County Water District and Cain-Papais Trust	R5-2008-0058	Acute, Chronic and Human Health	Stanislaus River
Ironhouse Sanitary District, Wastewater Treatment Plant	R5-2008-0057	Acute, Chronic and Human Health	San Joaquin River
Town of Discovery Bay, Discovery Bay Wastewater Treatment Plant	CA0078590	Acute and Chronic	Old River
City of Portola, Wastewater Treatment Plant	NPDES No.: CA0077844 Order #: R5-2009-0093	Acute & Chronic	Middle Fork, Feather River
City of Rio Vista, Beach Wastewater Treatment Facility	R5-2008-0108	Acute, Chronic and Human Health	Sacramento River

In summary, the information provided to the Regional Water Board previously and above supports the District's proposed mixing zone as it meets the requirements of the SIP and also satisfies USEPA guidelines for mixing zones that have been used in other states.

The Issue Paper also states that "ammonia levels in the Delta are a concern due to the toxicity of ammonia and the effect ammonia can have on dissolved oxygen." With respect to the applicability of these issues at the edge of the proposed mixing zones, it should be noted that modeling results presented by the District indicate that neither toxicity nor low dissolved oxygen levels would occur at these locations..

The Issue paper also states that "removal of ammonia is both technically feasible and commonly employed by most dischargers in the Central Valley Region." While this may be

[5] Region 5 Permits can be found at: http://www.swrcb.ca.gov/centralvalley/board_decisions/adopted_orders/

true, these dischargers referred to in the Issue Paper are not similar to SRCSD. In particular, the dischargers that have been required to remove ammonia in the Central Valley typically discharge to effluent dominated water bodies where there is limited or no dilution available. In contrast, the SRWTP discharges to the Sacramento River where significant dilution is available. Furthermore, technical feasibility should not be an overriding consideration when establishing effluent limits in an NPDES permit. As indicated in [Cost Benefit analysis dated May, 2010], the costs of nitrification are significant and should not be imposed on local communities unless information exists to indicate that a commensurate environmental benefit would be achieved. Available information, as summarized below, indicates that, beyond the ammonia reduction needed to prevent low dissolved oxygen in downstream waters, further ammonia reduction is not warranted or reasonable.

AMMONIA

Ammonia is the subject of ongoing studies to understand its role in the Delta ecosystem. Several statements regarding ammonia in the Issue Paper are not supported by the body of current research as discussed in detail below. The District's detailed comments are related to the following:

- Ammonia Toxicity
- Synergistic effects
- Inhibition of Phytoplankton Primary Production
- Shift in algal communities

In brief, the Issue Paper does not recognize recent findings regarding the occurrence, or lack thereof, of ammonia-based acute and chronic toxicity as stated in the May 2010 Central Valley Regional Water Quality Control Board Draft "Nutrient Concentrations and Biological Effects in the Sacramento-San Joaquin Delta" report. With regard to potential synergistic effects, ambient percentages of effluent in the Sacramento River just below the discharge are well below the no effects threshold for "percent effluent" obtained in Inge Werner's effluent dosing experiment. The environmental relevance of exposure concentrations has received less attention than deserved in investigations of contaminants in the Delta by some researchers. Several key elements of the ammonium inhibition hypothesis researched by Dugdale and Parker (San Francisco State University) were not confirmed by the Sacramento River study in 2009. Cecile Mioni's research (University of California, Santa Cruz), including data from sampling events in October 2008 and June-August 2009, has revealed a lack of correspondence between ammonium concentrations and toxic *Microcystis* blooms. Instead, independent studies in several Pelagic Organism Decline ("POD") years (2004, 2005, 2008, 2009) have consistently indicated that other factors such as water temperature, flow and turbidity best explain *Microcystis* abundance and toxicity in the Delta.

<u>Ammonia toxicity</u>

The statement, "*[a]mmonia is extremely toxic to aquatic life at low levels*," is not placed in sufficient context with the abundant recent research that indicates that ambient ammonia concentrations in the Sacramento River - and in the whole Delta as defined by the Issue Paper - are well below 1999 USEPA chronic or acute ammonia criteria *and* are well below concentrations which are currently estimated to be acutely toxic to sensitive Delta species such as Delta smelt and the calanoid copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi*. Examples from recent research are as follows:

USEPA Criteria (1999). The Issue Paper statement "*[s]tudies indicate that the Delta waters rarely exceed the USEPA ammonia acute or chronic criteria*" implies that the acute criterion is sometimes exceeded in the Delta. This is not true. Also exceedances of the chronic criterion are *extraordinarily* rare. A screening of almost 12,000 samples from 80 stations throughout the upper San Francisco Bay Estuary, collected over 35 years (1974-2010)[6] resulted in *zero* exceedances of the acute criterion, and *only two* exceedances of the chronic criterion[7]. Neither of the two exceedances of the chronic criterion occurred during the POD years of 2000-2010. Margins of safety (estimated by dividing USEPA criterion values for each sample by the corresponding ambient ammonia concentration) are very large for the Delta. Over the available time record, mean margins of safety for the acute criterion are 295 and 243 for freshwater and brackish sites, respectively[8]. Analogous margins of safety for the chronic criterion are 74 and 52. This topic is discussed in more detail in Attachment A1.

With respect to the recently released Draft USEPA Criteria (2009) for the protection of sensitive freshwater mussels, it is important to note that those draft criteria (which are referenced in the issue paper) are still under review and have not been finalized by USEPA. Thus, the draft criteria are not appropriate for use in NPDES permitting decisions at this time. Additionally, the presence of sensitive freshwater mussels near the SRWTP discharge has not been established or documented at this time.

Delta smelt. No measurements of ambient un-ionized ammonia thus far reported from the freshwater or brackish Delta have exceeded the LC50 or LC10 for Delta smelt larvae obtained in 7-day acute toxicity tests in 2009 (Werner et al. 2009)[9]. No ambient un-ionized ammonia concentrations reported during POD years (2000-2009) from freshwater stations have exceeded the NOEC reported by Werner et al. (2009) for 7-day survival tests (wherein ammonia was supplied via additions of SRWTP effluent).

Delta copepods. Although chronic toxicity test results for Delta copeopods are not yet available (the life cycle tests referred to in the Issue Paper), very large margins of

[6] The dataset and the screening are detailed in Engle, D.L., & G. Lau. 2009a. *Total and Un-ionized Ammonia Concentrations in the Upper San Francisco Estuary: A Comparison of Ambient Data and Toxicity Thresholds*. 9th Biennial State of the San Francisco Estuary Conference, Oakland, CA, September 29-October 1, 2009, and in Engle, D.L. (2010) (see below).

[7] The two exceedances occurred at IEP-EMP station C3 (Sacramento River at Greene's Landing) in October 1991, and at IEP-EMP station P8 (San Joaquin River at Stockton) in April 1976.

[8] Engle, D.L. (2010) Testimony before State Water Resources Control Board. Delta Flow Criteria Informational Proceeding. Other Stressors-Water Quality. Ambient ammonia concentrations: direct toxicity and indirect effects on food web. Avail. at: http://www.waterboards.ca.gov/waterrights/water_issues/programs/bay_delta/deltaflow/sac_rcsd.shtml

[9] Werner, I., L.A. Deanovic, M. Stillway, and D. Markiewicz. 2009. Acute toxicity of ammonia/um and wastewater treatment effluent-associated contaminants on Delta smelt - 2009. Final Report, submitted to the Central Valley Regional Water Quality Control Board, December 17, 2009.

safety between ambient ammonia levels in the Delta and acute thresholds for *Eurytemora affinis* suggest that chronic toxicity is an unlikely explanation for population trends for this species. *Median* ambient un-ionized ammonia concentrations in the freshwater and brackish Delta during POD years[10] were 100 and 166 times lower, respectively, than the 96-hr LC50 for *E. affinis* (0.12 mg N/L) published by Teh et al. (2009)[11]. The 99th percentile values for un-ionized ammonia during POD years[12] are more than an order of magnitude lower than the *E. affinis* LC50.

Synergistic effects

Referring to tests described in Werner (2009), the Issue Paper states "*there are indications that additive or synergistic effects are occurring in the SRWTP effluent where ammonia may be combining with other unknown toxicants resulting in toxicity...The study showed that the test performed with SRWTP effluent was statistically more toxic than the test performed with river water seeded with ammonium chloride. This may be an indication that there are additional toxicants present in the SRWTP effluent that are resulting in chronic toxicity to delta smelt.*"

It is not reasonable to conclude from the work of Werner et al. (2009) that synergistic effects would occur in the Sacramento River at the ambient ammonia levels downstream from the SRWTP discharge. The concentrations of SRWTP effluent that produced effects in these particular tests are significantly higher than the ambient concentrations existing below the SRWTP discharge. The 7-day effects thresholds in Werner et al. (2009) for 47-d old delta smelt, expressed as percent effluent, were as follows: LC50 (25.7%), LC10 (10.6%), NOEC (9%). In contrast, the percentages of effluent that occur in the Sacramento River below the SRWTP discharge are typically less than 3%[13]. In other words, ambient percentages of effluent in the Sacramento River just below the discharge are well below the *no effects* threshold for "percent effluent" obtained in Werner's effluent dosing experiment. The environmental relevance of ambient exposure concentrations has received less attention than deserved in investigations of contaminants in the Delta.

Inhibition of Phytoplankton Primary Production

The Issue Paper states "*It is unknown what the impact of ammonia is in the freshwater Delta between the SRWTP discharge and Suisun Bay*". The Issue Paper does not acknowledge recent research results which pertain to nitrogen/phytoplankton interactions between the

[10] 0.00072 and 0.0012 mg N/L (un-ionized ammonia-N), respectively for freshwater and brackish stations (calculated using the dataset described in Engle (2010)).

[11] 0.12 mg N/L (un-ionized ammonia), obtained at representative pH 7.6. Published in: Teh, S.J, S. Lesmeister, I. Flores, M. Kawaguchi, and C. Final Report. Acute Toxicity of Ammonia, Copper, and Pesticides to Eurytemora affinis, of the San Francisco Estuary. Appendix A In: Reece, C., D. Markiewicz, L. Deanovic, R. Connon, S. Beggel, M. Stillway, and I. Werner. 2009. *Pelagic Organism Decline (POD): Acute and Chronic Invertebrate and Fish Toxicity Testing in the Sacramento-San Joaquin Delta*. UC Davis Aquatic Toxicology Laboratory, Progress Report, 29 September 2009.

[12] 0.0063 and 0.014 mg N/L (un-ionized ammonia-N), respectively for freshwater and brackish station (calculated using the dataset described in Engle (2010)).

[13] Based on 7-day running averages for Sacramento River flow between 1998-2009, the 99.5th percentile percent effluent is 2.8% (M. Mysliwiec, Larry Walker Associates, unpublished data).

SRWTP and Suisun Bay. Since the August 2009 Ammonia Summit, results of detailed transect work in the Sacramento River between the SRWTP and Suisun Bay conducted by San Francisco State University (SFSU) investigators (Alex Parker, R. Dugdale, and others) have been presented at the 2009 State of the San Francisco Estuary Conference (Parker et al. 2009)[14] and in a draft report to the Regional Board[15], released in March 2010.

Several key elements of the ammonium inhibition hypothesis were not confirmed by the Sacramento River study referred to above. Grow-out tests showed that phytoplankton growth rates collapsed after ambient nitrate was depleted upstream of the SRWTP, whereas on the same time frame, phytoplankton growth was prolonged by ammonium uptake below the SRWTP. In the Sacramento River, specific uptake rates for ammonium were not lower than those for nitrate when ammonium was in abundance. Longitudinal patterns in biomass and primary production rates in the Sacramento River were *not* explained by ambient ammonium concentrations or differential uptake of ammonium and nitrate. Three results in particular illustrate that ammonium is not disrupting *in situ* primary production in the Sacramento River:

1. Carbon fixation rates declined along the river upstream of the SRWTP, despite the fact that nitrate dominated N uptake in that reach of the river.
2. No step-change in phytoplankton biomass or carbon fixation rates was associated with either (1) the location of the SRWTP discharge, or (2) a shift from predominantly nitrate uptake by phytoplankton to predominantly ammonia uptake below the discharge.
3. Significant increases in primary production rates occurred in the river between Rio Vista and Suisun Bay, despite the fact that inorganic nitrogen uptake in that reach was dominated by ammonium.

Finally, between the Yolo/Sacramento County line and Suisun/San Pablo Bays, small-celled phytoplankton and green algae exhibited similar longitudinal trends as large celled (presumably) diatoms. These observations so far refute the hypothesis that ammonium inputs create a competitive disadvantage for large diatoms compared to other taxa.

<u>Shift in algal communities</u>

The Issue Paper states: "*A hypothesis is that the elevated concentrations of ammonia in the Delta are responsible for shifting the competitive advantage to less nutritious bluegreen algae such as Microcystis in late summer...Microcystis abundance appears to be positively correlated with ammonium...*" A presentation given by Dr. Cecile Mioni (UCSC) at the August 2009 Ammonia Summit is cited as support for this hypothesis. However, as noted

[14] Parker A.E., R.C. Dugdale, F.P. Wilkerson, A. Marchi, J. Davidson-Drexel, J. Fuller, and S. Blaser. 2009. *Transport and Fate of Ammonium Supply from a Major Urban Wastewater Treatment Facility in the Sacramento River, CA*. 9th Biennial State of the San Francisco Estuary Conference, Oakland, CA, September 29-October 1, 2009.

[15] Parker, A.E., A.M. Marchi, J. Drexel-Davidson, R.C. Dugdale, and F.P. Wilkerson. 2010. Effect of ammonium and wastewater effluent on riverine phytoplankton in the Sacramento River, CA. Draft Final Report, submitted to the Central Valley Regional Water Quality Control Board, March 17, 2010.

in the District's letter regarding the Human Health Issue Paper (February 1, 2010), this presentation was based on preliminary, incomplete results from post-doctoral sampling work in the Delta in the summer of 2009. Subsequent analysis of more complete results from Dr. Mioni's research, including data from sampling events in October 2008, and June-August 2009, has revealed a lack of correspondence between ammonium concentrations and toxic *Microcystis* blooms. Dr. Mioni's more complete analysis was presented by her at several venues starting in late 2009 and more recently at the Oceans Colloquium at Hopkins Marine Station (April 23, 2010)[16], and the Delta Science Program Brown Bag Series in Sacramento (May 12, 2010)[17]. Among Dr. Mioni's current conclusions from her Delta research include the following:

- There is no apparent association between ammonium concentrations or NH_4^+:P ratios and either *Microcystis* abundance or toxicity.
- Water temperature is strongly correlated with *Microcystis* abundance and toxicity.
- Secchi depth and specific conductivity are likely correlated with *Microcystis* abundance and toxicity.

Regarding *Microcystis*, the Issue Paper states '*data collected to date is ambiguous*'. However, independent studies in several POD years (2004, 2005, 2008, 2009) consistently indicate that physical factors such as water temperature, flow, and turbidity best explain *Microcystis* abundance and toxicity in the Delta. While the Issue Paper acknowledges the work in Lehman et al. (2008),[18] which indicates that water temperature and low stream flow are positively linked to *Microcystis* abundance, it omits the additional result from this publication that ammonia was weakly *negatively* correlated with *Microcystis* abundance, meaning that higher ammonia concentrations were associated with fewer *Microcystis*. Finally, the lack of correspondence between ambient ammonia concentrations and the abundance of *Microcystis* in the Delta was recently confirmed in additional published work, Lehman et al. (2010)[19]:

> "Although ammonium-N concentration was elevated at some stations in the western and central delta and the Sacramento River at stations at CS and CV, neither it nor the total nitrogen (nitrate-N and nitrite-N plus ammonium- N) to soluble phosphorus molar ratio (NP) was significantly correlated with Microcystis abundance across all regions or within the western and central delta separately. Plankton group carbon or plankton species abundance at 1 m was not significantly correlated with any of the

[16] Mioni, C.E. (2010) *What controls harmful algae and phytotoxins in the SF Bay?* Oceans Colloquium, Hopkins Marine Station, Monterey, CA. April 23, 2010.

[17] Mioni, C.E., and A. Paytan (2010) *What controls Microcystis bloom & toxicity in the San Francisco Estuary? (Summer/Fall 2008 & 2009).* Delta Science Program Brownbag Series, Sacramento, CA. May 12, 2010.

[18] Lehman, P.W., G. Boyer, M. Satchwell, and S. Waller. 2008. The influence of environmental conditions on the seasonal variation of *Microcystis* cell density and microcystins concentration in the San Francisco Estuary. Hydrobiologia 600: 187-204.

[19] Lehman, P.W., S.J. Teh, G.L. Boyer, M.L. Nobriga, E. Bass, and C. Hogle. 2010. Initial impacts of *Microcystis aeruginosa* blooms on the aquatic food web in the San Francisco Estuary. Hydrobiologia 637: 229-248.

water quality conditions measured, including the NP ratio." (Lehman et al. 2010, p. 237).

An association between water temperature and and cyanobacterial blooms in the Delta would be consistent with observations from other estuaries. Increased residence time (e.g., during drought) and warmer temperatures are acknowledged as factors stimulating cyanobacterial blooms in other estuaries (Paerl et al. 2009[20], Paerl & Huisman 2008[21]).

Non-nutrient factors which affect the taxonomic composition of phytoplankton in estuaries have been neglected in the POD debate. For example, ammonium inhibition of nitrate uptake has received considerable attention as a hypothesized factor to explain changes in the relative abunance of diatoms in the estuary. However, physical factors (such as temperature, current speed, residence time, stratification, light penetration) may be strongly affecting competitive outcomes between diatoms and other phytoplankton taxa in the Delta, irrespective of nutrient concentrations or ratios. Published information indicating this is true is available for the Delta. Lehman (1996, 2000) associated a multi-decadal decrease in the proportional biomass of diatoms in the Delta and Suisun Bay to climatic influences on river flow. The deep, pool-like bathymetry of the Stockton Deepwater Ship Channel is hypothesized by some investigators to function as a trap for diatoms in transport in the San Joaquin River. Diatoms settle more rapidly than other taxa; unless current speeds are high, diatoms may not be able to remain in suspension for the length of the ship channel (P. Lehman, DWR, Feb. 2009, personal communication). The influence of flows and residence time on phytoplankton assemblages in estuaries is well acknowledged in other regions. For example, hydrologic perturbations, such as droughts, floods, and storm-related deep mixing events, overwhelm nutrient controls on phytoplankton composition in the Chesapeake Bay; diatoms are favored during years of high discharge and short residence time (Pearl et al. 2006)[22]. The role of flow and residence time in regulating estuarine microfloral composition was summarized by an expert panel convened by CalFed in March 2009. The panel's final document "*Ammonia Framework*" (Meyer et al. (2009)[23] states as follows:

> "Diatoms have fast growth rates and may be particularly good competitors during high flows with concomitant short residence times, when their fast growth rates can offset high flushing rates. In moderate flows, chlorophytes and cryptophytes become more competitive, whereas low flows with concomitant longer residence times allow the slower-growing cyanobacteria, non-nuisance picoplankton, and dinoflagellates to contribute larger percentages of the community biomass. These spatially and temporally-variable patterns of phytoplankton composition are typical of many

[20] Pearl, H.W., K.L. Rossignol, S. Nathan Hall, B.L. Peierls, and M.S. Wetz. 2009. Phytoplankton community indicators of short- and long-term ecological change in the anthropogenically and climatically impacted Neuse River Estuary, North Carolina, USA. Estuaries and Coasts. DOI 10.1007/s12237-009-9137-0

[21] Paerl, H.W., and J. Huisman. 2008. Blooms like it hot. Science 320: 57–58. doi:10.1126/science.1155398

[22] Pearl, H.W., L.M. Valdes, B.L. Peierls, J.E. Adolf, and L.W. Harding, Jr. 2006. Anthropogenic and climatic influences on the eutrophication of large estuarine ecosystems. Limnol. Oceanogr. 51(1, part 2): 448-462.

[23] Meyer, J.S., P.J. Mulholland, H.W. Paerl, and A.K. Ward. 2009. A framework for research addressing the role of ammonia/ammonium in the Sacramento-San Joaquin Delta and the San Francisco Bay Estuary Ecosystem. Final report submitted to CalFed Science Program, Sacramento, CA, April 13, 2009.

estuaries [e.g., Chesapeake Bay, Maryland; Neuse-Pamlico Sound, North Carolina; Narragansett Bay, Rhode Island; Delaware Bay, Delaware]." Meyer et al. (2009)

Benthic grazing may also be altering phytoplankton composition in the estuary. Clam grazing selectively removes larger particles (Werner & Hollibaugh 1993)[24]; and, clams may consume a larger fraction of diatoms than nanoplanktonic taxa such as flagellates. Kimmerer (2005)[25] used long-term dissolved silica dynamics, corrected for mixing in the low salinity zone, as an indicator of diatom productivity in the northern San Francisco Estuary. He showed that there was a step decrease in annual silica uptake after 1986, which he attributed to efficient removal of diatoms by *Corbula amurensis* after its introduction in 1986. Grazing by *Corbicula fluminea* can cause shallow habitats in the freshwater Delta to serve as a net sink for phytoplankton (Lopez at al. 2006; Parchaso & Johnson 2008)[26]; it is possible that diatoms are differentially affected by benthic grazing (e.g., compared to motile or buoyant taxa) in both the brackish and freshwater Delta. In fact, benthic grazing has been implicated as a factor favoring *Microcystis* over other phytoplankton, as explained in the CalFed expert panel's "*Ammonia Framework:*"

> "However, in places where filter-feeding mussels and clams overlap with habitat suitable for *Microcystis* (i.e., low salinity), the presence of these invertebrates might enhance bloom formation by selectively rejecting large *Microcystis* colonies. That grazer selectivity can give *Microcystis* a grazer-resistant, competitive advantage over other phytoplankton, as Vanderploeg et al. (2001) reported for zebra mussels (*Dreissena polymorpha*) in the Great Lakes." (Meyer et al. 2009)

Finally, the Issue Paper states that removal of ammonia and nitrate is '*technically feasible*' (pp.6, 10) and "*commonly employed by most dischargers in the Central Valley Region*' (p.6-7). It should be clarified that the primary reasons for including nitrification and denitrification facilities at Central Valley POTWs has typically been to meet water quality based effluent limitations pertaining to ammonia toxicity based on adopted, applicable U.S. EPA criteria and/or nitrate MCLs for POTWs with little or no dilution in their receiving waters. In no cases in the Central Valley have POTWs been required to install facilities to remove ammonia, nitrate or phosphorus compounds to address purported biostimulatory impacts or the other hypotheses addressed above.

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[24] Werner, I., and J. T. Hollibaugh. 1993. *Potamocorbula amurensis*: Comparison of clearance rates and assimilation efficiencies for phytoplankton and bacterioplankton. Limnol. Oceanogr. 38: 949-964.

[25] Kimmerer, W. J. 2005. Long-term changes in apparent uptake of silica in the San Francisco Estuary. Limnol. Oceanogr. 50: 793-798.

[26] Lopez, C.B., J.E. Cloern, T.S. Shraga, A.J. Little, L.V. Lucas, J.K. Thompson, and J. R. Burau. 2006. Ecological values of shallow-water habitats: implications for the restoration of disturbed ecosystems. Ecosystems 9: 422-440.

Parchaso F., and J. Thompson. 2008. *Corbicula fluminea* distribution and biomass response to hydrology and food: A model for CASCaDE scenarios of change. CALFED Science Conference, Sacramento, CA., October, 2008. Avail at http://cascade.wr.usgs.gov/CALFED2008.shtm

LOW DISSOLVED OXYGEN

The Issue Paper discusses ambient dissolved oxygen data downstream of the SRWTP discharge and approaches to preventing dissolved oxygen levels below the Basin Plan objective. Information regarding these two topics is provided in this section.

Dissolved Oxygen Data Evaluation

The Issue Paper states that several water quality databases include dissolved oxygen data sowing that the Sacramento River below the SRWTP has been 'at times out of compliance with the Basin Plan's dissolved oxygen water quality objective [of 7 mg/L] while the river upstream of the SRWTP is always in compliance.'

As noted in the Issue Paper, the District has evaluated the effect of the SRWTP effluent on downstream dissolved oxygen concentrations. Based on Regional Water Board comments, the District has made substantial additions to the original dissolved oxygen analysis. In the evaluation, the District recognized that the available data from the various data sources were, at times, inconsistent and contradictory. The District and USGS measure dissolved oxygen at Freeport. The next site with dissolved oxygen data downstream from the discharge is the Department of Water Resources (DWR) Hood station, 8 miles downstream. In comparing the District and USGS dissolved oxygen to the Hood dissolved oxygen data, the difference between the two location ranges from more than 1.0 mg/L to over 2.0 mg/L, including periods of high river flow conditions where little change in dissolved oxygen would be expected between the two sites (i.e, high flow provides short travel time with little opportunity for decay and high flows result in high levels of dilution minimizing any impact of the SRWTP effluent). The data for Freeport and Hood are presented in Figure 1. In the modeling analysis, the dissolved oxygen data at Hood could not be depressed using realistic reaction rates. It was the difference between the Freeport data and Hood data that caused the District to conduct a Dissolved Oxygen data assessment for all data sets.

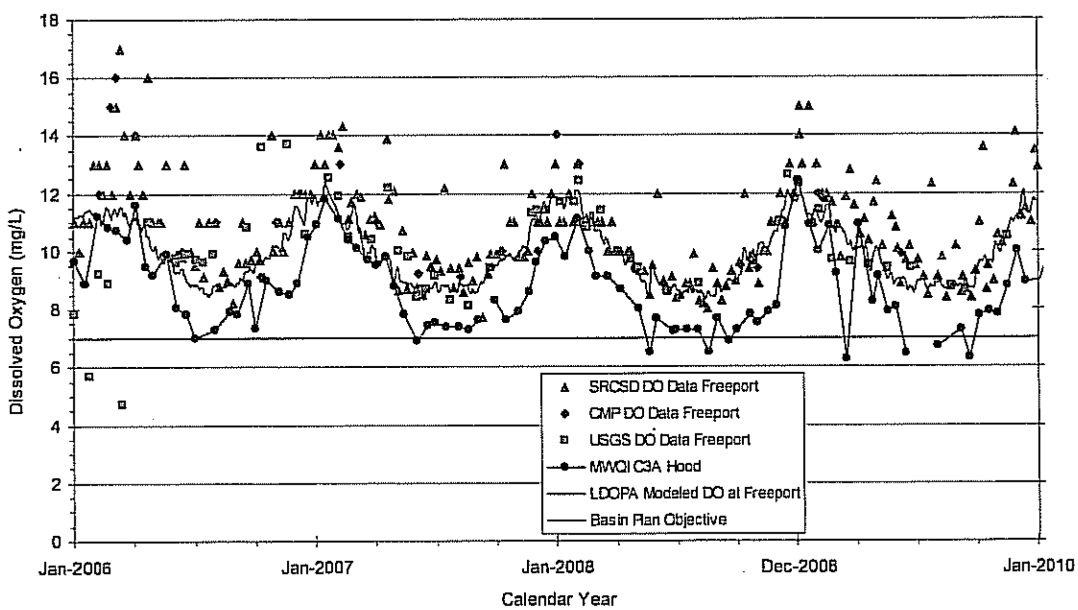

Figure 1: Dissolved Oxygen Concentration Comparison Between Freeport and Hood.

To further investigate the potential causes for inconsistencies between data sets, the District has been performing an ongoing data assessment of the available data sets, and methods used to collect the information. A summary of the programs collecting dissolved oxygen, the methods, and calibration is presented in Table 2. In the data assessment, the District is evaluating the available data, the quality assurance/quality control (QA/QC) procedures implemented by the programs, and the calibration records. Again, the data at Hood are not consistent with data collected at other locations on the River. The USGS and District dissolved oxygen data at Freeport are comparable. Likewise, the USGS and CDEC data at Rio Vista are also comparable. CDEC and MWQI data at Hood are comparable, however, there is no mechanism that can realistically account for the difference in dissolved oxygen concentrations between Freeport and Hood and Hood and Rio Vista. The data collected by the Regional Water Board[27] do not support a consistent dissolved oxygen sag between the point of SRWTP discharge and Hood. The Regional Water Board data does not support a sag that would then continue to deepen as the water continued downstream.

Table 2: Data Sources for Dissolved Oxygen in the Sacramento River.

	USGS	CMP	Regional Water Board	CDEC	City of Rio Vista
Site	Freeport and Rio Vista	Freeport and RM44	Multiple Sacramento River Locations	Hood and Rio Vista	Rio Vista
Field Meter Type and Model	YSI Multi-Parameter 600XL	YSI Multi-Parameter 600XL	YSI Multi-Parameter 556	YSI Clark/ Optical ROX[1]	YSI Multi-Parameter 550A
Method	Clark (amperometric)	Clark (amperometric)	Clark (amperometric)	Clark/ROX[1] (amperometric/ luminescent)	Clark (amperometric)
Time of Calibration	Morning of sampling event	Morning of sampling event	Morning of sampling event	Periodically[2]	-
Sample Location	Mid-Channel	Mid-Channel	Mid-Channel	Near Bank	Bank
Depth of Sample	1 to 2 feet	2-5 feet	-	1 meter	2 feet
Time of Sampling	Morning (10-12am)	Morning (10-12am)	-	Continuous (hourly)	-

[1] DWR changed sensor from Clark to optical in 2008
[2] Calibration schedule has not been provided

Additionally, the CDEC data are the uncorrected sensor reading and do not receive QA/QC. The data on CDEC are marked provisional and subject to change[28]. The District was able to

[27] Chris Foe, Adam Ballard and Stephanie Fong (2010), "Draft Nutrient Concentrations and Biological Effects in the Sacramento-San Joaquin Delta", May 2010

[28] http://cdec.water.ca.gov/faq.html#quality

review dissolved oxygen calibration results for the Hood station from March through December 2008[29]. In the period, there were three events (April 2, April 28, and December 10) where the dissolved oxygen was adjusted up by over 1.0 mg/L. The data in the CDEC database are not back corrected to account for the calibration adjustments. Due to concerns over the available data and the inconsistencies between programs and locations, the District concludes that the dissolved oxygen may be a field parameter that has not received the best quality control and care in measurement and recording. The District is especially concerned that the Regional Water Board would use the CDEC data directly in evaluations of the receiving water compliance with objectives due to the fact that they are provisional data and subject to change.

Reduction of Oxygen Demanding Substances

The Issue Paper states that the District is 'examining operational changes such as eliminating the high ammonia leachate from the sludge lagoons that is treated at the SRWTP.' The subject waste stream is mischaracterized as ammonia leachate. There has been no leachate collection and treatment from the SSBs. The ponds were flushed with treated effluent to control struvite production and odor, and returned to the headworks of the SRWTP. It is also a mischaracterization to say that the District is only examining operational changes. In fact, the SSB flushing was discontinued in May 2009 for 19 of 20 ponds and has resulted in an estimated 12% reduction in ammonia in the SRWTP effluent. The SRCSD is committed to further limiting oxygen demand in its effluent in order to maintain compliance with dissolved oxygen water quality objectives downstream of the discharge that may result at higher discharge rates in the future. Some of the alternatives that the District is considering are listed in the Low Dissolved Oxygen Prevention Assessment submitted to the Regional Board in May 2009 with an updated version submitted in May 2010 and include process optimization, treatment of internal return flows, expansion of the District's water recycling program, or treatment of a portion of the SRWTP effluent flow.

THERMAL CONDITIONS

The Issue Paper states that, since 2005, 'there has been a significant pelagic organism decline (POD), new species are threatened and there has been a change in the diffuser configuration.' Significant declines in the populations of the delta smelt and other species have been observed since 2000 with the steepest decline observed from 2000-2002;[30] however, water temperature in the Sacramento River has not been implicated as one of the direct contributions to the POD in any of the numerous studies evaluating stressors to Delta species. As discussed below, the SRWTP discharge has a negligible effect on temperatures in the lower Sacramento River upon fully mixing. Consequently, its contribution to Delta temperatures, and therefore any potential POD-related effects, is also negligible.

An assessment of the thermal effects of the SRWTP effluent plume on the aquatic community of the lower Sacramento River was recently conducted in support of the SRCSD's proposed Thermal Plan exceptions. This assessment was based on a dye study

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[29] Email communication between Mike Dempsey, DWR and Kathleen Harder CVRWQCB, Feb 25, 2009.

[30] Pelagic Organism Decline Progress Report: 2007 Synthesis of Results; available: http://www.fws.gov/sacramento/es/documents/POD_report_2007.pdf

conducted in November 2007 (i.e., following the closure of the 25 eastern-most ports on the diffuser), three-dimensional simulations of the near field thermal plume using the computational fluid dynamics model FLOWMOD, and predicted far-field fully mixed thermal conditions using the U.S. Bureau of Reclamation's PROSIM model.

This assessment indicates that the near-field conditions in the plume would not pose substantial adverse effects on the balanced, indigenous aquatic community of the lower Sacramento River. Under all conditions modeled, a zone of passage at least 75 feet wide, in which temperatures are unaffected or minimally affected by the SRWTP effluent, occurs on each side of the diffuser, thereby leaving an adequate zone of passage around the plume. Furthermore, the closure of 25 diffuser ports in 2007 increased the zone of passage along the east side of the river by approximately 100 feet. Because the diffuser lies on the bottom of the river, the warmest temperatures occur near the bottom at the point of discharge, and temperatures within the plume are rapidly attenuated as the effluent rises and mixes toward the surface downstream of the diffuser. Consequently, surface temperatures within the river are only minimally affected by the time the plume approaches the surface downstream of the diffuser. In no case would the plume be expected to cause a thermal barrier to fish movement.

Because the warmest part of the thermal plume is located close to the outfall on the bottom of the river, few fish are expected to be exposed to the maximum temperature differentials between the effluent and river background, and exposure to the thermal plume would occur for short (i.e., minutes) periods of time. As actively swimming fishes approach the diffuser, they can readily avoid unfavorable temperatures within the plume by swimming around or over the portions of the plume. Passively drifting fishes or benthic macroinvertebrates may drift through the plume; however, given the rate of river flow and their thermal tolerances, they would not experience exposures to elevated temperatures for a sufficient period of time to cause lethal or sub-lethal effects.

Far-field temperature modeling results indicate that the probability with which any given fully mixed Sacramento River temperature would occur would not change substantially whether the SRWTP is operated to meet the: 1) Thermal Plan objective 5.A.(1)a year-round; 2) the current exception to this objective in the District's 2000 NPDES permit; or 3) the proposed Thermal Plan exceptions. This is due to the relatively infrequent occurrence of temperature differentials (between the SRWTP effluent and river background) that exceed 20°F. Consequently, the findings of this far-field assessment are consistent with a finding that the proposed Thermal Plan exceptions would be protective of the balanced, indigenous aquatic community of the lower Sacramento River and Delta.

PYRETHROIDS

The Issue Paper cites a recent study by Weston[31] to identify sources of pyrethroid pesticides in the Sacramento- San Joaquin Delta. Regarding the findings of this study, the issue paper states that "...although minimal toxicity was detected in the Sacramento River, SRWTP effluent contained pyrethroid pesticides in concentrations that may be toxic."

[31] Weston, D.P., Lydy, M.J., "Urban and Agricultural Sourcesof Pyrethroid Insecticides to the Sacramento-San Joaquin Delta of California", Environmental Science and Technology 2010, 44, 1833-1840.

The environmental relevance of any pyrethroids in SRWTP effluent is an important consideration. Because the SRWTP never discharges if the river to effluent flow is below 14:1 the impacts of undiluted effluent are not environmentally relevant. The statement regarding effluent pyrethroid levels in the issue paper implies that SRWTP effluent could be contributing to 'minimal toxicity' in the Sacramento River. The implication is misleading for several reasons:

- Toxicity related to pyrethroids in the Sacramento River was observed by Weston from samples that were taken upstream of the SRWTP discharge. Therefore, the 'minimal toxicity' observed in the receiving water was due to pyrethroids that occur in the absence of SRWTP discharge.

- Weston and Lydy (2010) did not collect samples or evaluate the toxicity of Sacramento River water downstream of SRWTP. Therefore, implications that SRWTP was causing toxicity due to pyrethroids in the receiving water environment are not supported by this study. Low ambient concentration estimates in the Delta and downstream of SRWTP discharge are validated by the rare instances of *H. azteca* toxicity reported in only 2% of samples in 2006-2007 by Werner et al.[32] and in only 0.5 % of samples in 2008 reported by Reece et al. [33]

- The toxicity to *Hyalella azteca* reported by Weston and Lydy (2010) in SRWTP effluent grab samples was in undiluted (100 percent) effluent. However, the SRWTP effluent is highly diluted when discharged into the Sacramento River, and the presence of toxicity in an undiluted sample provides no evidence of toxicity in the receiving water environment. Accounting for dilution of the effluent, downstream ambient concentrations (as shown in Table 3) would be well below those that have the potential to cause effects (pyrethroid EC50s reported in Weston and Lydy [2010] ranged from 1.7 to 21.1 ng/L). Note that permethrin, the least toxic pyrethroid with an EC50 of 21.1 ng/L, accounted for 36 to 82 percent of the summed pyrethroid concentrations in samples where pyrethroids were detected. There is, therefore, very little potential for toxicity in the Sacramento River from any pyrethroids discharged in SRWTP effluent.

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[32] Werner I, Moran K. 2008. Effects of pyrethroid insecticides on aquatic organisms. In Gan J, Spurlock F, Hendley P, Weston D (Eds). Synthetic Pyrethroids: Occurrence and Behavior in Aquatic Environments. American Chemical Society, Washington, DC.

[33] Reece, C., D. Markiewicz, L.Deanovic, R. Connon, S. Beggel, M. Stillway, and I Werner, I.L.2009. Pelagic Organism Decline (POD): Acute and Chronic Invertebrate and Fish Toxicity Testing in the Sacramento-San Joaquin Delta 2008-2010, Progress Report III. 29 September

Table 3
Estimated Pyrethroid Concentrations in the Sacramento River based on SRWTP Effluent Concentrations (Weston Study Results).

Sample Date	Units	1/27/2008	5/27/2008	7/15/2008	7/15/2008 (duplicate)	9/19/2008	11/2/2008	2/17/2009
Conditions	-	WET	DRY	DRY	DRY	DRY	WET	WET
Effluent Flow	MGD	193.5	143.8	149.3	149.3	158.8	248.8	215.7
dilution	(:1)	94	47.7	59.6	59.6	39.2	33.6	95.4
bifenthrin	ng/L	0	(0.057)	0	0	0	0	0
lamda-cyhalothrin	ng/L	0.06	0	0.06	0.11	0	0	0
esfenvalerate	ng/L	0	0	0	0	0.094	0	0
deltamethrin	ng/L	0	0	0	0	0	0	0
permethrin	ng/L	0.07	0	0.20	0.24	0.44	0	0.10
cyfluthrin	ng/L	(0.018)	0	0	0	0	0	0
cypermethrin	ng/L	0	0	0	0	0	0	0.18
fenpropathrin	ng/L	0	0	0	0	0	0	0
Summed Pyrethroids	ng/L	*0.15*	*(0.057)*	*0.26*	*0.35*	*0.53*	*0*	*0.28*

Notes:
Values in brackets were based on qualified results.
Concentrations could range from 0-3 ng/L for non-detects in effluent samples; therefore summed pyrethroid concentrations in the river could range from 0 – 0.71 ng/L even when none are detected.

- *H. azteca* are extremely sensitive to pyrethroids (effects in the 1-20 ng/L range are reported in Weston and Lydy 2010). Effects to this invertebrate are not necessarily indicative of effects to any other organism. In fact, aquatic wildlife are essentially unaffected by pyrethroids until concentrations are orders of magnitude above those that affect invertebrates.[34] Effect levels for fish are also well above the effect levels for invertebrates and are in the 60 to 6200 ng/L range.[35]

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[34]Beavers JB, Hoxter KA, Jaber MJ. 1990. PP321: A one-generation reproduction study with the mallard (Anas platyrhynchos). USEPA MRID: 41512101.

Roberts NL, Phillips C, Anderson A, MacDonald I, Dawe IS, Chanter DO. 1986. The effect of dietary inclusion of FMC 54800 on reproduction in the mallard duck. FMC Study No: A84/1260. EPA MRID: 00163099

Fletcher DW. 1983. 8-day dietary LC50 study with FMC 54800 technical in mallard ducklings. FMC Study No: A83/966. MRID: 00132535

Carlisle JC, Toll PA. 1983. Acute dietary LC50 of cyfluthrin technical to mallard ducks study number 83-175-02. Mobay Environmental Health Research Corporate Toxicology Dept. Stilwell, KS. Study number 85937. CDPR ID: 50317-003

[35] Kent SJ, Shillabeer N. 1997a. Lambda-cyhalothrin: Acute toxicity to golden orfe (Leuciscus idus). ZENECA Agrochemicals. CDPR ID: 50907-085.

Kent SJ, Shillabeer N. 1997b. Lambda-cyhalothrin: Acute toxicity to the guppy (Poecilia reticulata). ZENECA Agrochemicals. CDPR ID: 50907-085.

Surprenant DC. 1991. Acute toxicity of FCR 4545 technical to Rainbow Trout (Oncorhynchus mykiss) under flow-through conditions. Miles Incorporated. Springborn Laboratories Inc. Wareham, MA. USEPA MRID: 45375002.

McAllister WA. 1988. Full life cycle toxicity of 14C-FMC 54800 to the fathead minnow (Pimphales promelas) in a flow-through system. FMC Study No: A86-2100. EPA MRID: 40791301

The Issue Paper goes on to state that "In every sample of the SRWTP, at least 70 percent of the organisms were dead or unable to swim. Pyrethroids were detected in 4 of 6 SRWTP samples."

This statement demonstrates the lack of a causal relationship between the observed toxicity and pyrethroids reported in SRWTP effluent ($r^2 = 0.004$). Toxicity was relatively constant among the SRWTP effluent samples while pyrethroid concentrations varied greatly (Table 4). Complete TIE testing was not conducted on all samples by Weston and Lydy (2010) and the relative proportion of toxicity to *H. azteca* in SRWTP effluent from pyrethroids is not clear. Further research to evaluate the occurrence and potential for pyrethroid toxicity in effluent and in the receiving water would be needed to determine if there is any potential for effluent pyrethroid levels to cause toxicity.

Table 4

Pyrethroid Concentrations in SRWTP Effluent Grab Samples (Weston Study Results).

Sample Date	Units	1/27/2008	5/27/2008	7/15/2008	7/15/2008 (duplicate)	9/19/2008	11/2/2008	2/17/2009
Conditions		WET	DRY	DRY	DRY	DRY	WET	WET
bifenthrin	ng/L	0	(2.7)	0	0	0	0	0
lamda-cyhalolhrin	ng/L	5.5	0	3.5	6.4	0	0	0
esfenvalerate	ng/L	0	0	0	0	3.7	0	0
delatamethrin	ng/L	0	0	0	0	0	0	0
permethrin	ng/L	7.0	0	12.2	14.2	17.2	0	9.4
cyfluthrin	ng/L	(1.7)	0	0	0	0	0	0
cypermethrin	ng/L	0	0	0	0	0	0	17
fenpropathrin	ng/L	0	0	0	0	0	0	0
Summed Pyrethroids	ng/L	*14.2*	*(2.7)*	*15.7*	*20.6*	*20.9*	*0*	*26.4*

Notes:

Values in brackets were qualified as estimated concentrations above the MDL but below the RL.

Concentrations could range from 0-3 ng/L for non-detects; therefore summed pyrethroids could range from 0 – 24 ng/L even when none are detected.

The Issue Paper states that Weston and Lydy (2010) "suggest at current flows, SRWTP discharges on average 9 grams per day (g/d) of pyrethroids in the dry season and 13 g/d during the wet season."

Weston and Lydy (2010) reported a "rough approximation" of the pyrethroid loading in the Sacramento River from SRWTP discharge. There is considerable uncertainty associated with this estimate that was understated in this publication. Detected pyrethroid concentrations reported in SRWTP effluent samples were quite variable among events, and for individual pyrethroids during each event (Table 4). Measured concentrations were also at or near reporting limits where the associated error is highest. Measurement error rates are demonstrated by the variable (±30%) ability of the analysis method to recover known quantities of pyrethroids spiked into quality assurance/quality control samples. Measurements were also based on single grab samples collected during each event and therefore provide little indication of the variability over various temporal scales. Load calculations compound these potential errors by multiplying concentrations by millions of liters discharged each day. Load estimates should include these uncertainties by reporting a range (i.e., 0 to 9 g/day) or an estimate of error (i.e., 9 ± 9 g/day) when discussing any calculated estimate.

The Issue Paper states that "at this time, the fate of the mass loading of pyrethroids from the SRWTP is unknown." Fate and transport play a key role in determining bioavailability and toxicity. Therefore, fate and transport must be considered in any assessment of pyrethroids. Factors that will affect the fate and transport of pyrethroids include:

- Pyrethroids are extremely hydrophobic and sorb strongly to particles and surfaces when in solution. The presence of suspended solids and sediments in samples greatly modifies and reduces bioavailability so that only the freely dissolved fraction exerts toxicity.[36] Therefore, the factors that affect bioavailability (e.g., organic carbon, suspended solids, dietary uptake, temperature) should be considered in any evaluations of potential toxicity. The potential for pyrethroid toxicity may be better estimated based on a measure of pyrethroids in the dissolved phase or from modeled bioavailable fractions.

- This tendency for pyrethroids to sorb to particles causes them to settle out of the water column and accumulate in the sediments. This transport mechanism will affect the media where pyrethroids are found and should be considered in evaluating pyrethroid fate and transport.

- Pyrethroids are largely degraded over a few weeks to months (20-60 day half-life) and do not accumulate in the environment (Laskowski, 2002).[37] This loss over time should also be considered in evaluations of pyrethroid fate, transport, and potential for toxicity.

Finally, the Issue Paper states that the 'Sacramento and San Joaquin Rivers were rarely toxic' which reinforces the contention that there is little evidence of pyrethroid concentrations in the SRWTP effluent having any environmentally relevant impact on receiving waters downstream of the discharge.

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[36] Amweg EL, Weston DP, Ureda NM. 2005. Use and toxicity of pyrethroid pesticides in the Central Valley, California, USA. Environ. Toxicol. Chem. 24:966-972

Day KE. 1991. Effects of Dissolved Organic Carbon on Accumulation and Acute Toxicity of Fenvalerate, Deltamethrin and Cyhalothrin to Daphnia magna (Straus). Environ. Toxicol. Chem. 10:91-101

Smith S, Lizotte RE. 2007. Influence of Selected Water Quality Characteristics on the Toxicity of l-cyhalothrin and g-cyhalothrin to Hyalella azteca. Bull. Environ. Contam. Toxicol. 79:548-551.

Yang WC, Gan JY, Hunter W, Spurlock F. 2006a. Effect of suspended solids on bioavailability of pyrethroid insecticides. Environ. Toxicol. Chem. 25:1585-1591

Xu YP, Spurlock F, Wang ZJ, Gan J. 2007. Comparison of five methods for measuring sediment toxicity of hydrophobic contaminants. Environ. Sci. Technol. 41:8394-8399

[37] Laskowski DA. 2002. Physical and chemical properties of pyrethroids. Rev. Environ. Contam. Toxicol. 174:49-170

WHOLE EFFLUENT TOXICITY

The Issue Paper discussed toxicity with respect to both acute and chronic toxicity assessments.

Acute toxicity

The Issue Paper states that "... recent flow-through bioassays conducted by SRCSD during regular effluent monitoring show intermittent toxicity, but the cause is unknown." The Issue Paper refers to violations of the requirement that no single bioassay may result in less than 70% survival and the requirement that the median result of consecutive bioassays may not be less than 90% survival.

SRCSD has spent considerable time and resources investigating possible sources of toxicity. To date, these investigations have not identified any toxicants that may be responsible for changes in effluent quality or any issues with maintenance or operations that may have contributed to toxicity. However, lower than average survival in control tanks could indicate that the quality of the fathead minnows used may be a contributing factor. The two violations with survivals less than 70% appear to be sporadic and the toxicity did not appear to be persistent. It is not unusual for a POTW to have intermittent toxicity from unknown causes and often it will go away without any specific treatment or process changes. Statistically speaking, the false positive rate for identifying toxicity based on the NOEC in non-toxic samples is 5 percent or 1 in 20.

With respect to the requirement that a median of three of any consecutive samples should not be below 90%, the SRWTP disagrees with Regional Water Board staff interpretation of this requirement. As noted in a letter to the V. Vasquez on February 10, 2010,[38]

> *"The permit language states that the median is calculated using "any three or more consecutive" test values. Since the 1985 permit, the SRWTP has been calculating and reporting the median on a monthly basis as is required by the EPA discharge self-monitoring report template. Our interpretation of the permit language is that the term "or more" was included to address variability in the number of weeks in a month and the intent was to apply the median calculation on a monthly basis. The self-monitoring report template only allows for one entry of the calculated median per month, supporting our interpretation that the median calculation is to be performed on a monthly basis. Based on our interpretation, there was one violation of this limit in November 2009, as reported in the self-monitoring reports.*

In addition, as stated in the February 10th letter,

- Extensive evaluation and additional sampling are conducted whenever low survival or a violation is experienced
- The SRWTP staff continue to investigate and evaluate the bioassay system and our procedures, including cause of low control survival rates

[38] Somavaru, P. 2010a. Letter to V. Vasquez, CVRWQB. 'Notice of Violation for Exceedances of the Acute Toxicity Bioassay Effluent Limitation, Sacramento Regional Wastewater Treatment Plant (SRWTP) (NPDES NO. CA 0077682, WDR Order No. R5-2000-0188). February 10, 2010.

- However, the frequency of testing (weekly) limits the extent to which system can be evaluated, as these investigation cannot be conducted when there is a test in progress
- Other than toxicity, compliance with effluent limits has been 100% since 2009
- Chronic WET testing for Fat Head Minnow have resulted with low chronic toxicity

The Issue Paper also refers to toxicity by unknown contaminants as identified by a UCD researcher.[39] It is important to consider that delta smelt acute toxicity testing with effluent-ammonia is now in its third year (2008-2010), and none of these smelt toxicity tests have showed toxicity at environmentally relevant concentrations of effluent or ammonia (Werner et al. 2009b; Werner et al. 2009c). To put this in context, toxicity by unknown contaminants was identified during a delta smelt bioassay in 2009, but no toxicity was identified in three other delta smelt bioassays conducted since 2008. In the one test conducted in 2009 that showed toxicity, effects were only significantly different from controls at ≥ 28 percent effluent (data from Werner et al. 2009c). This is 15-20 times greater than the effluent concentrations typically present in the Sacramento River. The two tests conducted in 2008 did not show any toxicity to delta smelt in up to 36 percent effluent, which is 18 times the typical concentration present in the Sacramento River (~2%). This effect has not demonstrated any persistence in SRWTP effluent by repeated observation and could have been an episodic event.

<u>Alternative Test Species</u>

The Issue Paper discusses the use of rainbow trout instead of fathead minnow as a test species for acute bioassays because it may be more applicable to Delta species. The EPA guidance document "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms"[40] provides a general description of the distribution, life cycle, and culture methods of fathead minnow and rainbow trout.

The native geographic range of rainbow trout is west of the Rocky Mountains and along the eastern Pacific Ocean, but the species has been widely introduced and established in cold water habitats worldwide. It thrives at temperatures between 3°C in the winter to 21 °C in the summer, with an optimum temperature between 10-16 °C. It can tolerate lower and higher temperatures if acclimated gradually (but cannot tolerate temperatures above 27 °C).

The fathead minnow is widely distributed in North America, and its ease of propagation as a bait fish has led to its widespread introduction within and outside its native range. The species is found in a wide range of habitats, abundant in muddy brooks, streams, creeks, ponds and small lakes. It is tolerant of high temperature and turbidity, and low oxygen concentrations.

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[39] Werner, I, "Effects of Ammonia/um and Other Wastewater Effluent Associated Contaminants on Delta Smelt", presented at the 18-19 August 2009 Ammonia Summit at the Central Valley Regional Water Quality Control Board.

[40] USEPA. 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. U.S. Environmental Protection Agency Office of Water, Washington DC. EPA-821-R-02-012.

Both species seem suitable for use in toxicity testing of Sacramento River water given their native temperature ranges. There does not appear to be any strong advantage to using rainbow trout over fathead minnow.

As shown in the Table 5, several Delta dischargers are currently required to use fathead minnow minnows as the acute toxicity test organism in their NPDES permits, with Stockton Wastewater Control Facility the only discharger required to use rainbow trout.

Table 5: Toxicity Test Species in Central Valley Permits

Facility	Permit adopted	WET Testing Requirements	
		Acute toxicity testing	Chronic toxicity testing
Manteca WWQCF	2009	Fathead minnow	Water flea, fathead minnow, green algae
Rio Vista Beach WWTF	2008; amended 2009	Fathead minnow	Water flea, fathead minnow, green algae
Modesto WQCF	2008	Fathead minnow	Water flea, fathead minnow, green algae
Stockton WWCF	2008	Rainbow trout	Water flea, fathead minnow, green algae
Tracy WWTP	2007	Fathead minnow or Rainbow trout	Water flea, fathead minnow, green algae

In addition to consideration of rainbow trout, the Issue Paper states that "It may also be appropriate to required [sic] additional acute toxicity testing using *Hyalella azteca*..."

H.azteca is a standard toxicity test organism that is commonly used for testing the toxicity of <u>sediment</u> (EPA 2000a, method 600/R-99-064). There are issues with the toxicity testing method for *H.azteca* in water only exposures that have been identified and described in the recent 2009 USEPA Draft Ammonia Criteria document. *H.azteca* is an epibenthic invertebrate which lives on the sediment surface at the interface between sediment and surface water. *H.azteca* is stressed when presented with habitat or test conditions where there is no substrate, such as in a glass toxicity-testing beaker. Acute WET testing within an NPDES permit should be conducted with one of the standard test species listed in the EPA's Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (EPA 2002a).[41] As listed in 6.1.2, these species are:

Freshwater Organisms

1. *Ceriodaphnia dubia* (daphnid)
2. *Daphnia pulex* and *D. magna* (daphnids)
3. *Pimephales promelas* (fathead minnow)
4. *Oncorhynchus mykiss* (rainbow trout) and *Salvelinus fontinalis* (brook trout)

[41] USEPA. 2002a. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Office of Water, Washington, DC. EPA-821-R-02-012.

H. Azteca is not included in toxicity testing requirements in any wastewater NPDES permits to date. In addition, *H. azteca* testing in the Delta has been extensive[42] in an effort to evaluate ammonia impacts. However, this testing has lead to inconclusive and varying results.[43]

Because of the existing problems with the test method and lack of precedent for use of this species for acute toxicity compliance determinations, the District strongly objects to the proposed use of *Hyallela azteca* as a test organism for acute testing in its NPDES permit.]

Hypothesis Testing vs. Point Estimate for Chronic Toxicity Testing

The Issue Paper states that "in situations where dilution has been allowed for chronic toxicity criteria, the point estimate may be a better method for determining compliance. The point estimate provides a more precise measurement of the magnitude of toxicity, which is needed when some level of effluent toxicity is allowed due to an approved mixing zone."

SRCSD agrees with this statement regarding the use of a point estimate for evaluating chronic toxicity testing results in the SRWTP NPDES permit. SRWTP effluent is highly diluted in the Sacramento River (the average hourly mean dilution ratio from 2006-2008 was 107:1); therefore, the point estimate is a relevant measure of toxicity for the SRWTP. In addition, there are other reasons why a point estimate measure of toxicity is a robust and safe method for use in NPDES permits that is more appropriate than hypothesis testing endpoints (i.e., the NOEC).

The use of NOEC in NDPES permitting has been criticized on statistical grounds by the scientific community.[44] In addition, EPA does not recommend its use for NPDES permitting,[45] and the European Organization for Economic Co-operation and Development

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42 Pelagic Organism Decline (POD): Acute and Chronic Invertebrate and Fish Toxicity Testing in the Sacramento-San Joaquin Delta 2006-2007.
http://www.science.calwater.ca.gov/pdf/workshops/POD/2008_final/Werner_POD2006-07Tox_Final_Report.pdf

43 Ammonia Summit, Various Presentations,
http://www.swrcb.ca.gov/rwqcb5/water_issues/delta_water_quality/ambient_ammonia_concentrations/index.shtml

44 Hoeven, N. van der, F. Noppert, and A. Leopold. 1997. How to measure no effect. Part I: Towards a new measure of chronic toxicity in ecotoxicology. Introduction and workshop results. Environmetrics 8: 241–248.

Chapman, P.M., R.S. Caldwell, and P.F. Chapman. 1996. A warning: NOECs are inappropriate for regulatory use. Environmental Toxicology and Chemistry 15 (2): 77-79

Kooijman, S. A. L. M. 1996. An alternative for NOEC exists, but the standard model has to be replaced first. Oikos 75: 310–316

Suter, G.W. 1996. Abuse of hypothesis testing statistics in ecological risk assessment. Human and Ecological Risk Assessment 2 (2): 331-347

Laskowski, R. 1995. Some good reasons to ban the use of NOEC, LOEC and related concepts in ecotoxicology. Oikos 73 (1): pp. 140–144

45 U.S. Environmental Protection Agency (EPA). 1991. Technical Support Document for Water Quality-based Toxics Control. U.S. EPA Office of Water. March. EPA/505/2-90-001

(OECD) concluded that the NOEC should not be used (OECD, 2006).[46] Rather, both tend to promote the use of point estimates as toxicity endpoints for NPDES permitting. EPA (1991) evaluated the merits and limitations of these endpoints and determined that the 25 percent inhibition concentration (IC25) is the preferred statistical method for determining toxicity endpoints. This standing was reaffirmed in the WET final rule where EPA stated:

> *"as previously stated in the method manuals (USEPA, 1993; USEPA, 1994a; USEPA, 1994b) and the USEPA's Technical Support Document (USEPA, 1991), USEPA recommends the use of point estimation techniques over hypothesis testing approaches for calculating endpoints for effluent toxicity tests under the NPDES Permitting Program[47]"*

The Fourth Edition chronic WET methods manual (EPA, 2002) further emphasized the use of point estimates (i.e., IC25) over hypothesis testing endpoints (i.e., the NOEC).

> *"NOTE: For the NPDES Permit Program, the point estimation techniques are the preferred statistical methods in calculating end points for effluent toxicity tests"* [48]

"The NOEC is an approximation of the no effect concentration (NEC) but is not a good estimate of this actual concentration at which no effect occurs." (Chapman, 1996) Instead, point estimates "use the concentration-response relationship to interpolate the precise effluent concentrations where significant toxic effects begin to occur" (SIP, 2005).

Multi-party written comments submitted to the SWRCB strongly support the use of point estimation procedures for evaluation of chronic toxicity test results for reasonable potential, trigger/limit derivation, and trigger/limit compliance.[49]

> *"The USEPA, as well as many experts in the field of toxicology, has long expressed a strong preference for the use of point estimation techniques*

U.S. Environmental Protection Agency (EPA). 1994. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Third Edition. July. EPA-600-4-91-002.

U.S. Environmental Protection Agency (EPA). 2000b. Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing (40 CFR Part 136). Office of Water. EPA 821-B-00-004. July.

USEPA. 2002a. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Office of Water, Washington, DC. EPA-821-R-02-012.

[46] Organization for Economic Co-operation and Development (OECD). 2006. Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application. Joint meeting of the chemicals committee and the working party on chemicals, pesticides, and biotechnology. Environmental Directorate. ENV/JM/MONO(2006)18. OECD Series on Testing and Assessment. Number 54

[47] 67 Fed. Reg. 69958 (November 19, 2002)

[48] U.S. Environmental Protection Agency (EPA). 2002b. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Fourth Edition. October. EPA-821-R-02-013

[49] Tri-TAC, CASA, BACWA, CVCWA, and SCAP. 2006. Comments on the Informational Document for Proposed Revisions to the Toxicity Control Provisions of the Policy of Implementation of Toxic Standards for Inland surface Waters, Enclosed Bays, and Estuaries of California. Letter to Regina Linville, State Water Resources Control Board. Dated 17 January.

(e.g., EC25/IC25) rather than hypothesis test procedures for compliance monitoring in the WET program. These recommendations are based upon a number of toxicological and statistical limitations of hypothesis test results, particularly when used in a compliance setting. In fact, since its inception, the acute toxicity program has successfully used effect-based statistics (i.e. LC50 or percent effect) for compliance determination. Use of point estimates to measure chronic toxicity is embracing the best science available and would demonstrably improve the consistency, reliability, and accuracy of the WET program within the State without any loss of environmental protection. Therefore, we urge the SWRCB to be consistent with these recommendations and join the growing number of State programs that use point estimates to regulate chronic toxicity."

The NOEC endpoint was never validated with field or laboratory test comparisons by EPA during interlaboratory testing (EPA, 2001)[50]. NOEC data were not part of EPA's Interlaboratory Variability Study (2001). Accordingly, the NOEC is not part of the adopted 40 CFR 136 Table 1A methods and should not be used for NPDES purposes (NACWA, 2006).[51] The NACWA (2006) white paper is found in Attachment A2 and provides additional discussion on the appropriateness of hypothesis and point estimates of toxicity.

It is also difficult to quantify the precision of NOEC endpoints between tests. In practice, the precision of results of repetitive chronic tests is considered acceptable if the NOECs do not vary by more than one concentration interval above or below a central tendency. This "acceptable" range is potentially very large and could vary from 6.25- to 25-percent effluent (4 to 16 NOEC TUc for tests using a 0.5 dilution factor; EPA, 2002b). When a substantial difference in toxicity endpoints exists between the NOEC and IC25, EPA concludes that the bioassay had a poorly defined dose-response curve and that results from these tests should be interpreted carefully. Additional dilution concentrations should be tested when there is a high degree of separation between the IC25 and NOEC endpoints.

The NOEC must be bounded by other test concentrations to be equal to one of the tested effluent concentrations. If an effect is observed in the lowest effluent concentration, then the test is inconclusive for the NOEC and it is reported as "less than" the lowest effluent dilution (e.g., NOEC <6.25%; >16 TUc).

Another drawback to results based on hypothesis testing is that most of the data is not used in the statistical analysis. The only data needed for the final result are the measured endpoints of the control treatment and no effect treatment. The variability between treatments (concentration-by-test interaction variance) is not considered in calculations to determine hypothesis-testing endpoints.[52] Furthermore, the statistical procedure protects

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[50] U.S. Environmental Protection Agency (EPA). 2001. Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods, Vol. 1. Office of Water . EPA 821-B-01-004. September.

[51] National Association of Clean Water Agencies (NACWA). 2006. Whole Effluent Toxicity (WET) NPDES Permit Testing and Limitations for Public Agencies. White Paper. January.

[52] Dhaliwal, B.S., R.J. Dolan, C.W. Batts, and J.M. Kelly. 1997. Warning: replacing the NOECs with point estimates may not solve regulatory contradictions. Environmental Toxicology and Chemistry 16 (2): 124–126

against drawing the wrong conclusion when a treatment has no effect (Type I Error or alpha), but gives little protection against drawing the wrong conclusion when the treatment does have an effect (i.e., low power [1-α]; Chapman et al., 1996). Point estimates use all WET test data to calculate a point (the test effluent concentration) on a regression to identify a concentration that causes a specific level of response.

A point estimate measure of toxicity can be considered "safe" to the receiving water because EPA (1991) considers an IC25 the approximate analogue of the NOEC. This conclusion was validated by Norberg-King (1991)[53] in 23 effluent and short-term chronic reference toxicant data sets for the fathead minnow, *Pimephales promelas*, and *C. dubia* where the reported IC25s were comparable to the NOECs.

As recognized by EPA (2000b), the NOEC is an unreliable measure under concentration-response relationships. An alternative toxicity endpoint analogous to the NOEC that is not hindered by the statistical limitation of hypothesis testing toxicity endpoints, and is supported by EPA (2002b), is a reasonable and safe alternative for an NPDES reporting basis for SRWTP toxicity tests. A point estimate measure of toxicity such as the IC25 would achieve this goal.

Use of Synthetic Dilution Water

As noted in the Issue Paper, the use of synthetic dilution water is allowed and is consistent with recently adopted Central Valley permits. The District also supports the use of synthetic dilution water.

The choice of what should be used as the dilution water is at the discretion of the permitting authority as EPA does not require that any single source be used (EPA 2002a):

> *"...no single dilution water type is required for all tests. The method manuals now clarify that receiving waters, synthetic waters, or synthetic waters adjusted to approximate receiving water characteristics may be used for dilution water, provided that the water meets the qualifications for an acceptable dilution water. EPA clarified in the method manuals that an acceptable dilution water is one which is appropriate for the objectives of the test; supports adequate performance of the test organisms with respect to survival, growth, reproduction, or other responses that may be measured in the test (i.e., consistently meets test acceptability criteria for control responses); is consistent in quality; and does not contain contaminants that could produce toxicity. EPA also provided clarification on the use of dual controls. When using dual controls, the dilution water control should be used for determining the acceptability of the test and for comparisons with the tested effluent. If test acceptability criteria (e.g., minimum survival, reproduction, or growth) are not met in the dilution water control, the test must be repeated on a newly collected sample. Comparisons between responses in the dilution water control and in the culture water control can be used to determine if the dilution water, which may be a receiving water, possesses ambient toxicity."*

[53] Norberg-King, T.J. 1991. Calculations of ICp Values of IC15, IC20, IC25, IC30, and IC50 for Appendix A of the Revised Technical Support Document. Memorandum to M. Heber, EPA

According to EPA, the use of river water under certain conditions is arguably inappropriate for conducting WET tests (EPA 2000):

> *"If the objective of the test is to determine the toxicity of an effluent in the receiving system, a local receiving water is recommended for use as dilution water* ***provided that the receiving water meets specific criteria****. The receiving water should be collected as a grab sample from upstream or near the final point of effluent discharge, have adequate year-round flow, support adequate performance of the test organisms,* ***be consistent in quality****, be free of contaminants that would produce toxicity and be free from pathogens and parasites that could affect WET test results. If the local receiving water fails to meet any of these criteria for use, a synthetic dilution water adjusted to approximate the chemical characteristics of the receiving water is recommended."*

Regional studies conducted on the Sacramento River show that instream toxicity upstream of the SRWTP discharge location has been documented in recent years. The State of California and Sacramento River Watershed Program (SRWP) have been conducting studies on the toxicity of ambient water in the Sacramento River Watershed since 1999 (Larsen and Connor 2002; List et al. 2002; SRWP 2003).[54] These studies have focused on algae (*Raphidocelis subcapitata*, also known as, *Selenastrum capricornutum*) and *Ceriodaphnia dubia*.

Numeric vs. narrative toxicity limit

The District agrees with the current State Board position (as reflected in the 2005 SIP) of using a narrative chronic toxicity limit with a numeric trigger for monitoring and further evaluation. This approach is consistent with other Central Valley permits. The Issue Paper states that the 'numeric trigger will be reconsidered in the permit renewal' with one option being to calculate maximum daily and average monthly triggers instead of a single trigger. The current numeric trigger in the SRWTP permit is >8 TUC which would correspond to dilution of 7:1, a value which does not occur downstream from the SRWTP. This value is more stringent than in other recently adopted Central Valley permits for discharges for which there is an acute and chronic mixing zone established which provides dilution credit as shown in Table 6.

A numeric trigger that would be consistent with the permits shown in Table 6 would be based on dilution at critical low flows. Dilution at critical low river flows (i.e., 1Q10 or 5400 cfs) would be 16:1 based on an effluent flow of 218 mgd which would correspond to a numeric trigger of 17:1.

54 Larsen, K. and V.M. Connor. 2002. Algae Toxicity Study Monitoring Results: 2000-2001. Regional Water Quality Control Board, Central Valley Region, California Environmental Protection Agency.

List, K., K. Larsen, and B. Stafford. 2002. Sacramento River Watershed Program Toxicity Testing Data Summary: 1999-2000. Regional Water Quality Control Board, Central Valley Region, California Environmental Protection Agency.

Sacramento River Watershed Program (SRWP). 2003. 2001-2002 Annual Monitoring Report (Public Draft). Sacramento River Watershed Program. http://www.sacriver.org

TABLE 6 - NUMERIC TOXICITY TRIGGERS (TUC) FOR ADOPTED NPDES PERMITS WITH ADOPTED ACUTE AND CHRONIC MIXING ZONES

Discharger	Order #	Numeric Monitoring Trigger Chronic Toxicity Unit (TUc) where TUc = 100/NOEC[55]	Acute/Chronic Dilution Credit
City of Chico, Chico Water Pollution Control Plant	R5-2010-0019	>10 TUc	47:1
City of Yuba City, City of Yuba City Wastewater Treatment Facility	R5-2007-0134-01 (as amended by Order No. R5-2010-0007)	>12 TUc	12:1
City of Angels, City of Angels Wastewater Treatment Plant	R5-2007-0031-01 (as amended by Order No. R5-2009-0074)	16 TUc	18:1 (Chronic) 9:1 (Acute)
Forest Meadows Wastewater Reclamation Plant, Calaveras County Water District and Cain-Papais Trust	R5-2008-0058	>25 TUc	67:1
Ironhouse Sanitary District, Wastewater Treatment Plant	R5-2008-0057	>16 TUc	20:1 (acute) 28:1 (chronic
Town of Discovery Bay, Discovery Bay Wastewater Treatment Plan	R5-2008-0179	10 TUc	13:1 (acute) 23:1 (Chronic)
City of Portola, Wastewater Treatment Plant	R5-2009-0093	20 TUc	20:1
City of Rio Vista, Beach Wastewater Treatment Facility	R5-2008-0108	>16 TUc	20:1

[55] NOEC = No Observed Effect Concentration

Testimony before State Water Resources Control Board
Delta Flow Criteria Informational Proceeding

Other Stressors-Water Quality: Ambient Ammonia Concentrations: Direct Toxicity and Indirect Effects on Food Web

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February 16, 2010

My name is Diana Engle and I am providing this testimony regarding hypothesized direct and indirect effects of ammonia on the pelagic ecosystem of the upper San Francisco Estuary. I am an aquatic ecologist with over 20 years of experience evaluating the ecology and biogeochemistry of lakes, streams, large rivers and floodplains, estuaries, and wetlands. My education includes a doctorate in ecology from the University of California at Santa Barbara. I have authored extensive assessments of water quality in coastal and inland environments of California, and have published peer-reviewed articles on topics including floodplain nutrient dynamics and carbon cycling, watershed mass balances and stream export, and the ecology of aquatic macrophytes, floodplain algae, and riverine and lacustrine zooplankton. More detailed biographical information may be found in the Statement of Qualifications which accompanies this written testimony.

I am actively involved in forums addressing the POD and ammonia-related issues in the Delta. I have been a member of the IEP POD Contaminants Work Team since early 2008, and was an invited panel member at both the March 2009 CalFed *Ammonia Workshop* and the Central Valley Regional Board's August 2009 *Ammonia Summit*. I was an invited speaker at the October 2009 IEP Workshop: *Bay-Delta Monitoring Questions & Tools for the 21st Century*, and presented a comprehensive analysis of ambient ammonia data from the San Francisco Estuary at the *9th Biennial State of the San Francisco Estuary Conference* in September 2009.

1. Summary and Purpose of Testimony

Hypothesized effects of ammonia in the ecosystem of the upper San Francisco Estuary fall into two main categories:

- Direct effects on fish or invertebrates owing to acute or chronic toxicity
- Indirect effects of ammonia on the pelagic food web, via alterations of phytoplankton biomass or quality

The purpose of this testimony is to highlight key studies and findings which address whether ammonia is a direct or indirect determinant of biomass or species composition of pelagic organisms in the upper San Francisco Estuary (SFE)[1]. Section 2 provides an overview of research from the SFE regarding key issues related to the hypotheses above. Sections 3-6 provide more detailed discussion of evidence for selected issues. Attachments 1-4 contain supplemental material, and are referenced in the text.

There is now considerable agreement that ambient ammonia levels throughout the estuary are not acutely toxic to fish or their invertebrate prey, including Delta smelt and key calanoid copepod species. Hypotheses related to other direct or indirect effects of ammonia are being addressed by ongoing research. However, to date, information emerging from these research activities does not support an argument that ammonia is significantly contributing to the pelagic organism decline (POD) or to undesirable changes in the estuarine food web. *Consequently, there is no compelling need for information about ambient ammonia concentrations to influence a determination of the volume and timing of Delta exports and other Delta flow criteria.*

2. Overview of Scientific Evidence that Should be Considered by the SWRCB

Direct Toxicity. Ample evidence indicates that ambient ammonia concentrations throughout the upper SFE are not high enough to cause acute toxicity to Delta smelt or to the wide range of aquatic organisms explicitly protected by current USEPA ammonia criteria. In addition, preliminary tests in 2009 using calanoid copepods from the Delta (which are prey items for Delta smelt) indicated that ambient acute toxicity is highly unlikely for these organisms at prevailing ammonia and pH levels. This characterization of ambient conditions applies not only to "POD" years (e.g., 2002 onward), but also to the entire 35-year period for which long-term monitoring data are available. The characterization also applies to the reach of the Sacramento River below the discharge of the Sacramento Regional Wastewater Treatment Plant (SRWTP) (e.g., River Mile 44 and points downstream).

Three principal lines of evidence are currently available which indicate a lack of acute ammonia toxicity in the SFE:

1. ***Screening of ambient concentrations using USEPA ammonia criteria.*** A comprehensive compilation of publicly available data from long-term water quality monitoring programs currently allows comparison of USEPA acute and chronic criteria with ambient ammonia concentrations in almost 12,000 grab samples taken throughout the freshwater and brackish estuary from 1974 to the present. Ammonia concentrations have *never* exceeded the USEPA acute criterion; the chronic criterion was exceeded *only twice* in the available record (in 1976, 1991). Margins of safety are large: on average in the freshwater Delta, the acute and chronic criteria exceed ambient concentrations by factors of 300 and 80, respectively. <u>This analysis shows that ambient concentrations of ammonia throughout the estuary, including in the Sacramento River below the SRWTP, have always met USEPA ammonia criteria by comfortable margins of safety.</u>
   - ⇒ More detailed information about the dataset referred to above, procedures used to screen the data using USEPA criteria, and results for data through January 2010, are presented in Section 3 below.

[1] The upper San Francisco Estuary is used herein to refer to the legal Delta, Suisun Bay, and eastern San Pablo Bay.

2. ***Acute toxicity testing using Delta smelt***. The Central Valley Regional Water Quality Control Board (Regional Board) has funded several rounds of acute toxicity tests using Delta smelt, conducted by the UC Davis Aquatic Toxicology Laboratory (UCD-ATL; Werner et al. 2009a, b). Tests have been conducted using larval Delta smelt (47- and 51-day old) and juveniles (149-day old), and using both 96-hr and 7-day exposure periods. Both ammonium chloride and Sacramento Regional Wastewater Treatment Plant (SRWTP) effluent have been used as sources of ammonia in exposure tests. Depending on the test, endpoints (e.g., LC50, LC10, LOEC, NOEC) have been expressed in one or more of the following terms:

    - total ammonia (the analytical measurement)
    - un-ionized ammonia (the calculated fraction of total ammonia which is toxic, which is pH and temperature dependent)
    - percent SRWTP effluent (which can be compared to the dilution factors which occur in the Sacramento River below the SRWTP discharge)

    Testing indicates that Delta smelt have similar acute sensitivity to ammonia as rainbow trout. This is significant because the USEPA acute criterion for ammonia which applies to water bodies with salmonids was specifically derived to protect rainbow trout. The testing has also revealed that ambient concentrations which occur in the freshwater and brackish estuary are well below acute effects thresholds for Delta smelt.

    ⇒ Published effects thresholds for Delta smelt are compared to ambient ammonia data in Section 4 below.

3. ***Preliminary acute toxicity tests with Delta copepods.*** During summer 2009, acute exposure tests were conducted using two calanoid copepods which are important prey items for Delta smelt (*Eurytemora affinis* and *Pseudodiaptomus forbesi*). Preliminary acute thresholds were presented at the Regional Board Ammonia Summit (Teh et al. 2009a) for three test pHs: 7.2, 7.6, 8.1. Comparison of these effects thresholds with ambient pH and ammonia concentrations from the estuary currently indicate that acute toxicity is highly unlikely for these copepods.

    ⇒ Published effects thresholds for *Eurytemora affinis* (Teh et al. 2009b) are compared to ambient ammonia data in Section 4 below.

Recent use of acute-to-chronic ratios (ACRs) to infer chronic toxicity in the Delta. Evidence that acute ammonia toxicity is not a key concern in the Delta has spurred interest in estimating chronic toxicity thresholds for selected Delta species which are not included in the USEPA database. Chronic test procedures are not available for Delta smelt. Chronic exposure tests for the calanoid copepod *Pseudodiaptomus forbesi* (life cycle tests), funded by the Regional Board, were planned between December 2009-April 2010; preliminary results were not available at the time of this writing. In the meantime, ACRs are being used by several investigators, in lieu of chronic toxicity test results, to postulate that ambient concentrations of ammonia in the Delta may be causing chronic toxicity to sensitive Delta species. Recently, this approach has been applied to Delta smelt in a manner which is not consistent with USEPA derivation of ACRs and which supports assumptions about chronic toxicity that may not be warranted.

⇒ A brief discussion of concerns regarding how this approach has been applied to Delta smelt is provided in Section 5 below, and supported by more detailed information in *Attachment 3*.

Contaminant Mixtures. Information is currently lacking about whether ambient concentrations of other contaminants in the Delta affect sensitivity of organisms to ammonia. Test results reported in Werner et al. (2009b) (in which effects thresholds for delta smelt were higher in exposure tests using ammonium chloride than in those using SWRTP effluent) have entered the larger discussion of potential effects of contaminant mixtures. However, the concentrations of SRWTP effluent (as percentages of total flow in the river) that produced effects in these particular tests are well out of the range produced by the SRWTP discharge. The 7-day effects thresholds in Werner et al. (2009b) for 47-d old delta smelt, expressed as percent effluent, were as follows: LC50 (25.7%), LC10 (10.6%), NOEC (9%). In contrast, the percentages of effluent that occur in the Sacramento River below the SRWTP discharge are less than 3% the vast majority of the time[2]. <u>The environmental relevance of exposure concentrations has received less attention than deserved in investigations of lethal or sublethal effects of ammonia and other contaminants in the Delta.</u>

Studies Related to Indirect Effects of Ammonia on the Food Web

Ammonium Inhibition. Published work from field surveys and microcosm experiments has shown that, under certain conditions, ambient ammonium concentrations above ~4μM delay uptake of nitrate and development of diatom blooms in Suisun, San Pablo, and Suisun Bays (Dugdale et al. 2007, Wilkerson et al. 2006). This phenomenon, termed "ammonium inhibition" by the principal investigators, has been added to the list of factors that may be affecting the base of the pelagic food web in the SFE, and is currently being investigated in the freshwater Delta.

During 2008-2009, research addressing the relationship between ammonium, nitrate, and phytoplankton growth rates focused on the Sacramento River. Multiple transect studies were conducted between fall 2008 and spring 2009 by Regional Board staff and by researchers from San Francisco State University. To date, the results of this work are not yet publicly available as reports or in peer-reviewed literature. However, some of the results were presented at the Regional Board's Ammonia Summit (Foe et al. 2009, Parker et al. 2009a) and at the 2009 State of the San Francisco Estuary Conference (Parker et al. 2009b).

<u>Several key elements of the ammonium inhibition hypothesis were not confirmed by the Sacramento River studies.</u> Longitudinal patterns in biomass (of several taxa) and primary production rates were *not* explained by ambient ammonium concentrations or differential uptake of ammonium and nitrate. In incubations of river water, phytoplankton grew as well in water enriched with ammonium as they did in water enriched with nitrate. Significant increases in primary production rates occurred in the river between Rio Vista and Suisun Bay, despite the fact that inorganic nitrogen uptake in that reach was dominated by ammonium. This new information led principal investigators to conclude:

> "It is unclear from these data what drives declines in primary production of chl-a [in the Sacramento River]." (Parker et al. 2009b)

⇒ More information about ammonium inhibition studies and results is provided in Section 6.

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[2] Based on 7-day running averages for Sacramento River flow between 1998-2009, the 99.5th percentile percent effluent is 2.8%.

Microcystis. Toxic blooms of the colonial form of *Microcystis aeruginosa* have occurred in the north SFE during summer months (June-November) since 1999, and are the only recorded toxic phytoplankton blooms in the northern SFE to date. There is speculation, primarily based on information from highly eutrophic estuaries or laboratory work outside the Delta, that ammonia levels in the Delta might be contributing to the occurrence or toxin-production of *Microcystis*. However, field studies of *Microcystis* from the Delta do not confirm a relationship between ambient ammonia levels and the abundance or toxicity of *Microcystis*. Instead, physical factors such as water temperature, flow, and turbidity appear to best explain *Microcystis* abundance and toxicity in the SFE. Lack of a positive association between *Microcystis* and ammonia concentrations has been found in three separate studies in the estuary.

1. Lehman et al. (2008). Canonical analysis was performed on bi-weekly data for 17 environmental factors, *Microcystis aeruginosa* cell abundance, and microcystin cell content, from a sampling program in the freshwater and brackish estuary in 2004. East side stream-flow, Contra Costa Canal pumping, and water temperature were the primary factors explaining the abundance and microcystin content of *Microcystis* in the brackish and freshwater reaches of the Delta. Ammonia and nitrate concentrations were weakly *negatively* correlated with *Microcystis* abundance, meaning that higher ammonia and nitrate concentrations were associated with fewer *Microcystis*.

2. Lehman et al. (2010). Bi-weekly sampling throughout the estuary in late summer 2005 revealed no association between *Microcystis* abundance and ammonium-N or N:P ratios:

> "Although ammonium-N concentration was elevated at some stations in the western and central delta and the Sacramento River at stations at CS [Cache Slough] and CV [Collinsville], neither it nor the total nitrogen (nitrate-N and nitrite-N plus ammonium- N) to soluble phosphorus molar ratio (NP) was significantly correlated with Microcystis abundance across all regions or within the western and central delta separately. Plankton group carbon or plankton species abundance at 1 m was not significantly correlated with any of the water quality conditions measured, including the NP ratio." (Lehman et al. 2010, p. 237).

3. Cecile Mioni (CALFED post-doctoral study in progress). At the Regional Board Ammonia Summit, Cecile Mioni presented partial results from post-doctoral sampling work in the Delta in the summer of 2009 (Mioni & Paytan, 2009) which led to remarks in her presentation that *Microcystis* abundance appeared to be positively correlated with ammonium. However, subsequent analysis of more complete results from Dr. Mioni's research, including samples from October 2008, and June, July, August 2009, revealed a lack of correspondence between *Microcystis* cell abundance and ammonium concentrations. The lack of correspondence between *Microcystis* cell abundance and ammonium was particularly evident for sites where *Microcystis* was producing toxin. Based on the more recent analysis, Dr. Mioni now concludes that water temperature and secchi depth are more strongly correlated with *Microcystis* abundance than ammonium concentrations (the results of the study are currently in preparation for publication):

> "As you will see, the NH4 vs Microcystis abundance relationship does not appear to be very strong when we add the August 2009. I am seeing a stronger correlation with the water temperature and the secchi depth." (pers. comm. from C. Mioni to D. Engle, Dec. 16, 2009)

Overall Quality of the Phytoplankton Assemblage. An observed shift in phytoplankton community composition from dominance by diatoms to increasing dominance by other, mostly smaller, taxa including miscellaneous (green) phytoflagellates, and the recent occurrence of blooms of *Microcystis*, underly a hypothesis that the quality of the phytoplankton assemblage as food for zooplankton is decreasing in the estuary. In turn, there is speculation that ammonium concentrations - or shifting N:P ratios - may be contributing to the observed shifts in species composition.

Non-nutrient factors affecting diatom abundance in the SFE are rarely discussed. Lehman (1996, 2000) attributed a multi-decadal in the proportional biomass of diatoms in the Delta and Suisun Bay to climatic influences on river flow. Clam grazing selectively removes larger particles (Werner & Hollibaugh 1993); clams may consume a larger fraction of diatoms that nanoplanktonic taxa such as flagellates. Kimmerer (2005) used long-term dissolved silica dynamics, corrected for mixing in the low salinity zone, as an indicator of diatom productivity in the northern SFE. He showed that there was a step decrease in annual silica uptake after 1986, which he attributed to efficient removal of diatoms by *Corbula amurensis* after its introduction in 1986. Diatoms settle more rapidly than other taxa. The deep, pool-like bathymetry of the Stockton Deepwater Ship Channel is hypothesized by some investigators to function as a trap for diatoms in transport in the San Joaquin River; unless current speeds are very high, diatoms cannot remain in suspension for the length of the ship channel (P. Lehman, DWR, Feb. 2009, personal communication). The extent to which shifts in diatom abundance in the SFE is explained by benthic grazing, or interannual variation in freshwater flows remains unanswered in the Delta.

Regarding phytoplankton quality, Regional Board staff concluded in a summary of the August 2009 Ammonia Summit:

> "Finally, due to the lack of data on phytoplankton community composition, there is no consensus yet demonstrating that elevated ammonia levels in the Delta have caused a shift in the algal community from diatoms to less nutritious forms." (Foe 2009)

Also lost from the food web discussions are several studies from the SFE which indicate that non-diatom organisms occupy an important position at the base of the pelagic food web, and that detritus-based pathways for energy transfer may contribute more to the pelagic food web in the Delta than has been acknowledged. This information is important because it argues for a more holistic framework for understanding productivity than the "diatom→copepod→fish" paradigm that drives much of the POD discussion. Such information led the Interagency Ecological Program to make the following acknowledgement in its 2007 Synthesis of Results:

> "...it is possible that the hypothesis that the San Fransisco Estuary is driven by phytoplankton production rather than through detrital pathways may have been accepted too strictly." (Baxter et al. 2008)

Examples of pertinent findings are:

- Gifford et al. (2007): Several zooplankton species in the SFE can shift between consumption of phytoplankton and consumption of heterotrophic microbes. In feeding experiments using natural plankton assemblages from the SFE, a cladoceran (*Daphnia*), a calanoid copepod *Acartia*, and two cyclopoid copepods (*Oithona davisae* and *Limnoithona tetraspina*), all grazed heterotrophic ciliates at higher rates than diatoms.
- Bouley & Kimmerer (2006): Significant grazing on heterotrophic ciliates was observed for both the filter-feeding calanoid copepods *Pseudodiaptomus forbesi* (a common Delta smelt prey item) and *Eurytemora affinis*.

- Hall & Mueller-Solger (2005): *E. affinis* and *P. forbesi* were more successfully cultured in the lab when fed the motile cryptophyte alga *Cryptomonas* than when fed the diatom *Skeletonema* or the green alga *Scenedesmus* suggesting these calanoid copepods might prefer motile prey.
- Rollwagen-Bollens & Penry (2003): The diet of *Acartia* spp. (an important calanoid copepod genus in the estuary) in San Pablo Bay was dominated by heterotrophic prey (especially protozoans such as ciliates and non-pigmented flagellates).

3. Comparisons of Ambient Ammonia Data with USEPA Criteria

Available Data. In water, ammonia primarily occurs as two forms: ammonium ion (NH_4^+) and un-ionized ammonia (NH_3). The sum of un-ionized ammonia and ammonium is commonly referred to as *total ammonia*. The un-ionized form (dissolved ammonia gas) is toxic to aquatic animals at concentrations which vary widely among taxa. Ammonium and un-ionized ammonia occur in an equilibrium ($NH_4^+ \leftrightarrow NH_3 + H^+$) which is affected by pH, temperature, and salinity. When measurements of total ammonia are accompanied by measurements of water temperature, pH, and either electrical conductivity (EC) or salinity, ambient concentrations of un-ionized ammonia can be calculated. Calculation of USEPA ammonia criteria also requires data for water temperature, pH, and (for saltwater samples) salinity or EC.

Publicly available, co-occurring measurements of total ammonia, pH, temperature, and EC from the last 35 years (1974-2010) are available from 80 long-term monitoring stations in the upper SFE. This dataset allows calculation of un-ionized ammonia concentrations (and USEPA criterion concentrations) for almost 12,000 ambient water samples obtained as monthly or bi-weekly grab samples. A breakdown of these stations by sampling entity is provided in Table 1. The location of these stations, and sample counts per station for the entire record, are illustrated in Figure 1. A detailed inventory of stations and sample counts is provided as *Attachment 1*.

Procedure. The dataset described above was screened for exceedances of applicable current USEPA-recommended acute and chronic ammonia criteria for freshwater (USEPA 1999) and saltwater (USEPA 1989). These criteria are designed to protect the most sensitive fish and aquatic invertebrate species for which acceptable test results are available. Criteria are revised periodically when new data become available and are vetted by the EPA[3]. In the USEPA databases which supported the 1999 freshwater and 1989 saltwater criteria development, the most sensitive freshwater species is rainbow trout and the most sensitive saltwater species is winter flounder. Owing to higher acute sensitivity of salmonids, compared to other fish taxa, and higher chronic sensitivity of early life stages of fish, compared to older fish, USEPA (1999) recommends different versions of the freshwater acute and chronic criterion depending on whether these sensitive taxa or life stages are present in a waterbody. For the screening exercise described herein, the more conservative "salmonids present" acute criterion and the "early life stages of fish present" chronic criterion were used. Formulas for calculating the criteria, and other formulas used in the screening procedure, are provided in *Attachment 2*.

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[3] In December 2009, USEPA released a draft update of freshwater ammonia criteria (USEPA 2009) for public comment.

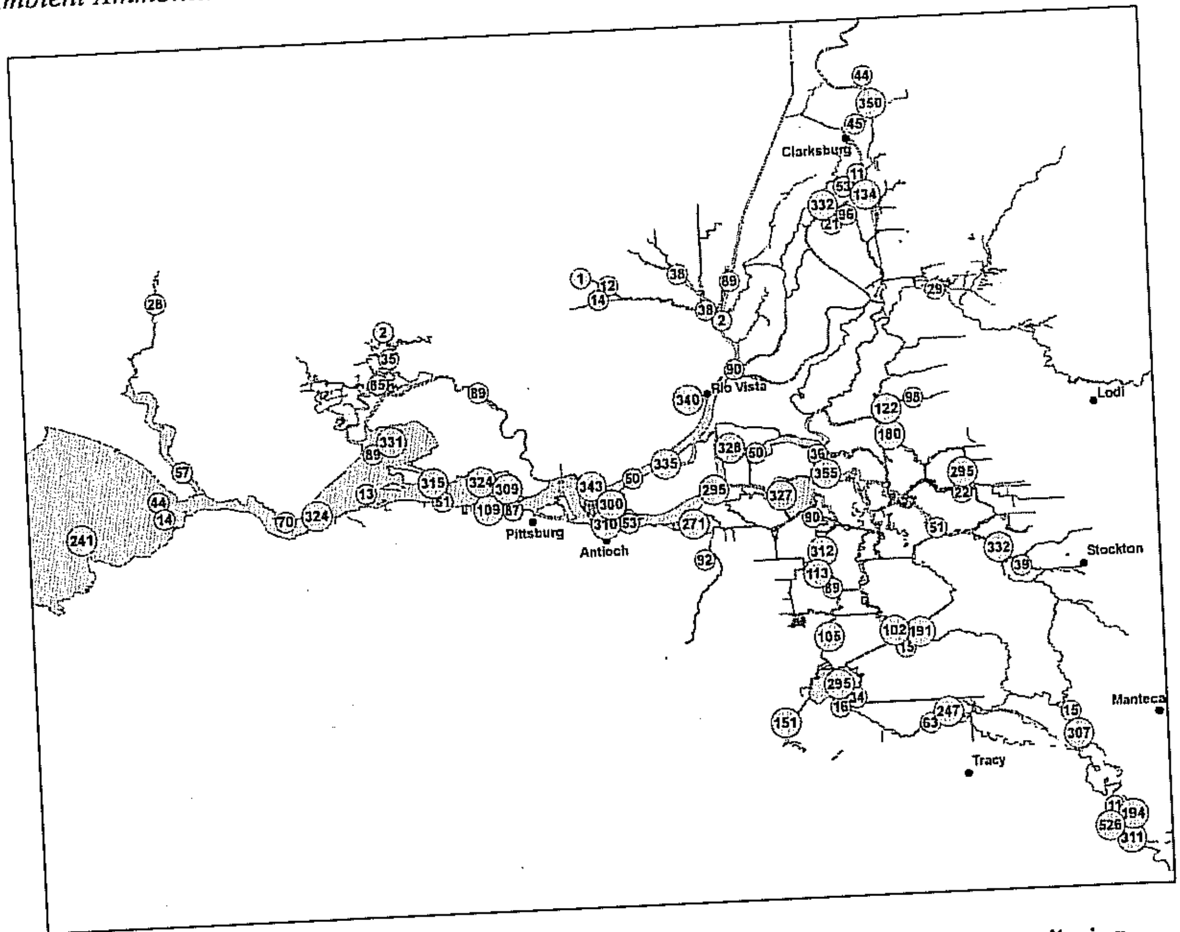

Figure 1. Long-term estuarine (green symbols) and freshwater (yellow symbols) monitoring stations in the Upper San Francisco Estuary providing co-occurring measurements of pH, water temperature, and total ammonia. Values inside symbols are numbers of monthly or bi-weekly grab samples taken during the period 1974-2010. Stations were classified as estuarine or freshwater based on procedures in the California Toxics Rule (see text). Monitoring programs are identified in Table 1.

Table 1. Availability of Co-Occurring Measurements of Total Ammonia, pH, Water Temperature, and Salinity/Electrical Conductivity in the Freshwater and Brackish Delta from Long-term Monitoring Programs in the Upper San Francisco Estuary[(1)].

Monitoring Program	Estuarine Reaches[(2)]				Freshwater Reaches[(2)]			
	1974-2010		"POD" era 2000-2010		1974-2010		"POD" era 2000-2010	
	Stations	**Samples**	**Stations**	**Samples**	**Stations**	**Samples**	**Stations**	**Samples**
California Department of Water Resources, Municipal Water Quality Investigations (DWR-MWQI)	1	109	1	85	14	917	7	656
Interagency Ecological Program Environmental Monitoring Program (IEP-EMP)	15	3840	5	49	22	4610	5	74
Acute and Chronic Invertebrate and Fish Toxicity Testing in the Sacramento-San Joaquin Delta, UC Davis Aquatic Toxicology Laboratory (UCD ATL POD Investigation)	12	625	12	625	11	663	11	663
US Geological Survey (USGS)	--	--	--	--	4	974	3	291
Sacramento Regional County Sanitation District Coordinated Monitoring Program (SRCSD-CMP)	--	--	--	--	2	89	2	89
Total	28	4574	18	759	53	7253	28	1773

(1) As used herein, the term upper San Francisco Estuary includes the legal Delta, Suisun Bay, Suisun Marsh, and eastern San Pablo Bay. Stations are illustrated in Figure 1.
(2) Stations were classified as estuarine (brackish) or freshwater using long-term records of salinity and the procedure outlined in the California Toxics Rule (CTR; USEPA 2000). Stations were classified as follows: "freshwater" if salinity was ≤1 ppt in ≥ 95% of samples, "saltwater" if salinity was ≥ 10 ppt in ≥ 95% of samples, and "estuarine" if salinity was between 1-10 ppt in ≥ 95% of samples.

Salinity is variable at the "estuarine" stations in the dataset. The California Toxics Rule (CTR) provides guidance on when freshwater versus saltwater objectives are applicable in estuarine water bodies (USEPA 2000, Section G2). Following the procedure outlined in the CTR for priority toxicants, exceedances at estaurine stations were determined as follows:

1. Both the Freshwater and Saltwater criterion was calculated for each ambient sample using ambient pH, temperature and salinity.
2. For each sample, the stricter (lower) criterion concentration was compared to the ambient concentration of total ammonia.[4,5]

Results. The results of the screening indicate that ambient ammonia concentrations meet USEPA acute and chronic criteria by comfortable margins of safety throughout the upper SFE. <u>For data spanning the period 1974-2010 (a total of 11,827 samples), the screening resulted in zero exceedances of the acute criterion, and only two exceedances of the chronic criterion</u>[6]. Neither of the two exceedances of the chronic criterion occurred during POD years. Based on the numbers of samples available for freshwater stations only, State Listing Policy (SWRCB 2004)[7] would require 622 exceedances for the period 1974-2010, or 101 exceedances for the period 2000-2010, to justify a 303(d) listing for ammonia toxicity in the Sacramento-San Joaquin Delta.

Margins of safety can be estimated by dividing USEPA criterion values (expressed as total ammonia) for each sample by the corresponding ambient concentration of total ammonia. Margins of safety obtained using this ratio are summarized in Table 2. The analysis indicates that, on average, the acute criterion exceeds ambient ammonia concentrations in the upper SFE by factors between 200-300. On average, the chronic criterion exceeds ambient concentrations by factors ranging from 40-80. Ample separation between ambient ammonia concentrations in the Sacramento River near Hood and acute and chronic criteria (calculated per sample based on ambient pH and temperature) is illustrated by the time series in Figure 2.

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[4] At estuarine stations, the freshwater acute criterion was stricter than the saltwater acute criterion for ~90% of samples, but the saltwater chronic criterion was stricter than the freshwater chronic criterion for ~80% of samples.

[5] Normally chronic criteria apply to 4-day averages of ambient concentrations (in saltwater), or 30-day averages of ambient concentrations (freshwater), not to monthly grabs. In absence of long-term monitoring data collected more frequently than monthly or bi-weekly, an underlying assumption of the screening exercise is that grab samples represent 4-day or 30-day average concentrations.

[6] The two exceedances occurred at IEP-EMP station C3 (Sacramento River at Greene's Landing) in October 1991, and at IEP-EMP station P8 (San Joaquin River at Stockton) in April 1976.

[7] The State Listing Policy (SWRCB 2004) contains procedures for determining how many exceedances of a particular Basin Plan objective must be observed before a water body can be placed on the 303(d) list as impaired by a given constituent or parameter. The procedure is based on the total number of measurements available from a water body, and the number of exceedances contained in the overall data set. The State Listing Policy procedure for toxicants involves using the binomial distribution to calculate the number of exceedances for which the probability of Type 1 and Type 2 error are minimized for an acceptable exceedance proportion of ≤ 3% and an unacceptable exceedance proportion of 18%.

Table 2. Mean Margins of Safety Separating US EPA Criterion Concentrations from Ambient Ammonia Concentrations in the Upper San Francisco Estuary

	Mean Margin of Safety (Criterion/Ambient Concentration)			
	Using Acute Criterion		Using Chronic Criterion	
	1974-2010	2000-2010	1974-2010	2000-2010
Freshwater Stations	295	312	74	80
Estuarine Stations	243	185	51	40

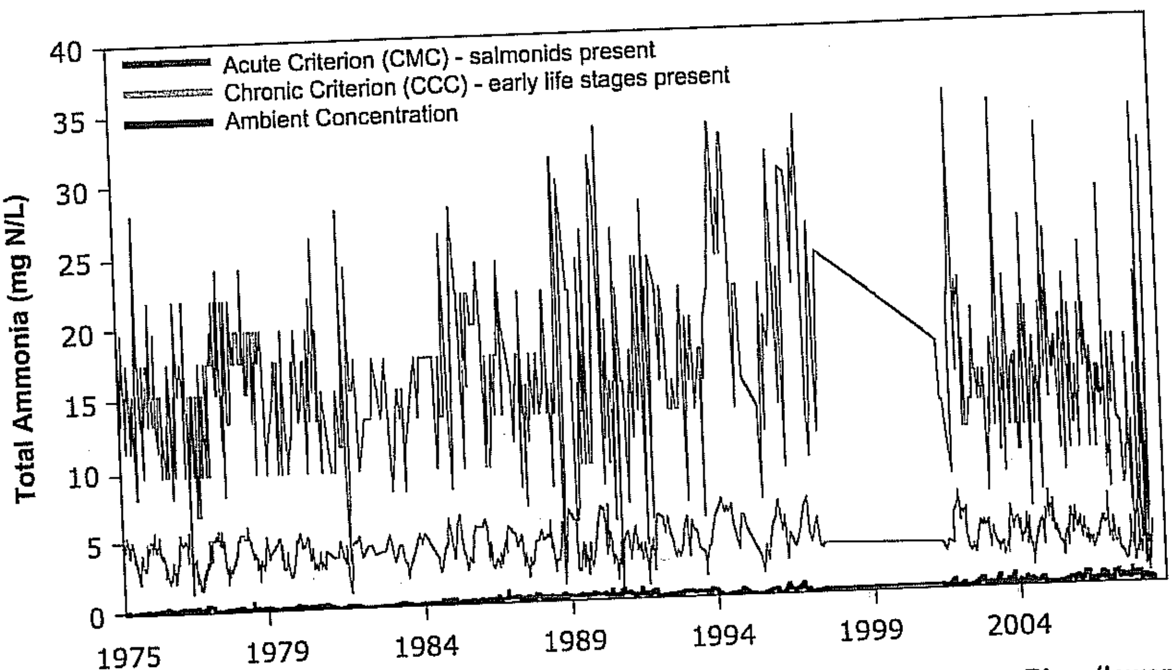

Figure 2. Comparison of ambient total ammonia concentrations in the Sacramento River (lower blue line) with the USEPA acute criterion (upper green line) and chronic criterion (middle red line). Data are from stations located at River Mile 44, Hood, and Greene's Landing.

4. Comparison of Ambient Ammonia Data with Effects Thresholds for Delta smelt and the Copepod *Eurytemora affinis*

Preliminary acute effects thresholds for ammonia, obtained in exposure tests using ammonium chloride, have been reported for larval and juvenile Delta smelt (Werner 2009), and one of its important prey items, the calanoid copepod *Eurytemora affinis* (Teh et al. 2009); thresholds expressed as un-ionized ammonia concentrations, are summarized in Table 3[8].

Table 3. Acute Effects Thresholds for Ammonia for Delta smelt and *Eurytemora affinis* from Exposure Tests using Ammonium Chloride.

Test Organism	Effects Threshold		References
	Threshold Type	**Un-ionized Ammonia (mg N/L)**	
larval Delta smelt (47-day old)	96-hr LC10	0.084, 0.105	Werner et al. (2009b) p. 15, 19
	96-hr LC50	0.164	
	7-day LC10	0.094	
	7-day LC50	0.113	
larval Delta smelt (51-day old)	96-hr LC10	0.096	Werner et al. (2009b) p. 17
	96-hr LC50	0.147	
juvenile Delta smelt (149-day old)	96-hr LC10	0.400	Werner et al. (2009b) p. 21
	96-hr LC50	0.557	
	7-day LC10	0.398	
	7-day LC50	0.515	
Calanoid copepod *Eurytemora affinis*	96-hr LC10 (pH 7.6)	0.08	Teh et al. (2009b)
	96-hr LC 50 (pH 7.6)	0.12	

In Figure 3, the ranked distributions of un-ionized ammonia concentrations for POD years for the freshwater and estuarine datasets, including the 99th percentile values, are compared to the lower effects thresholds for Delta smelt and *Eurytemora affinis* in Table 3. The comparison indicates that a significant margin of safety separates ambient ammonia concentrations in the upper SFE from acute effects thresholds so far reported for these two species.

[8] Copepod sensitivity varied inversely with pH in tests by Teh et al. (2009b). For example, the 96-hr LC10 and LC50 obtained at a test pH of 7.2 were 0.011 and 0.068 mg N/L, respectively. Analogous values for a test pH of 8.1 were 0.46 and 0.78 mg N/L. Thresholds presented in Table 3 are for the test pH which best represents ambient conditions in the upper SFE. Using the dataset described in this document, median and mean pH for estaurine stations for 2000-2010 are 7.7 and 7.6, respectively. Median and mean pH for freshwater stations for 2000-2010 both equal 7.6. Between 1974-2010, un-ionized ammonia concentrations exceeded the *lowest* LC10 (0.011 mg N/L) in Teh et al. (2009b) in only six samples with less-than-median pH (pH $\leq$ 7.6). This indicates that comparison of ambient ammonia concentrations with the copepod test results obtained at the median pH is a reasonable approach.

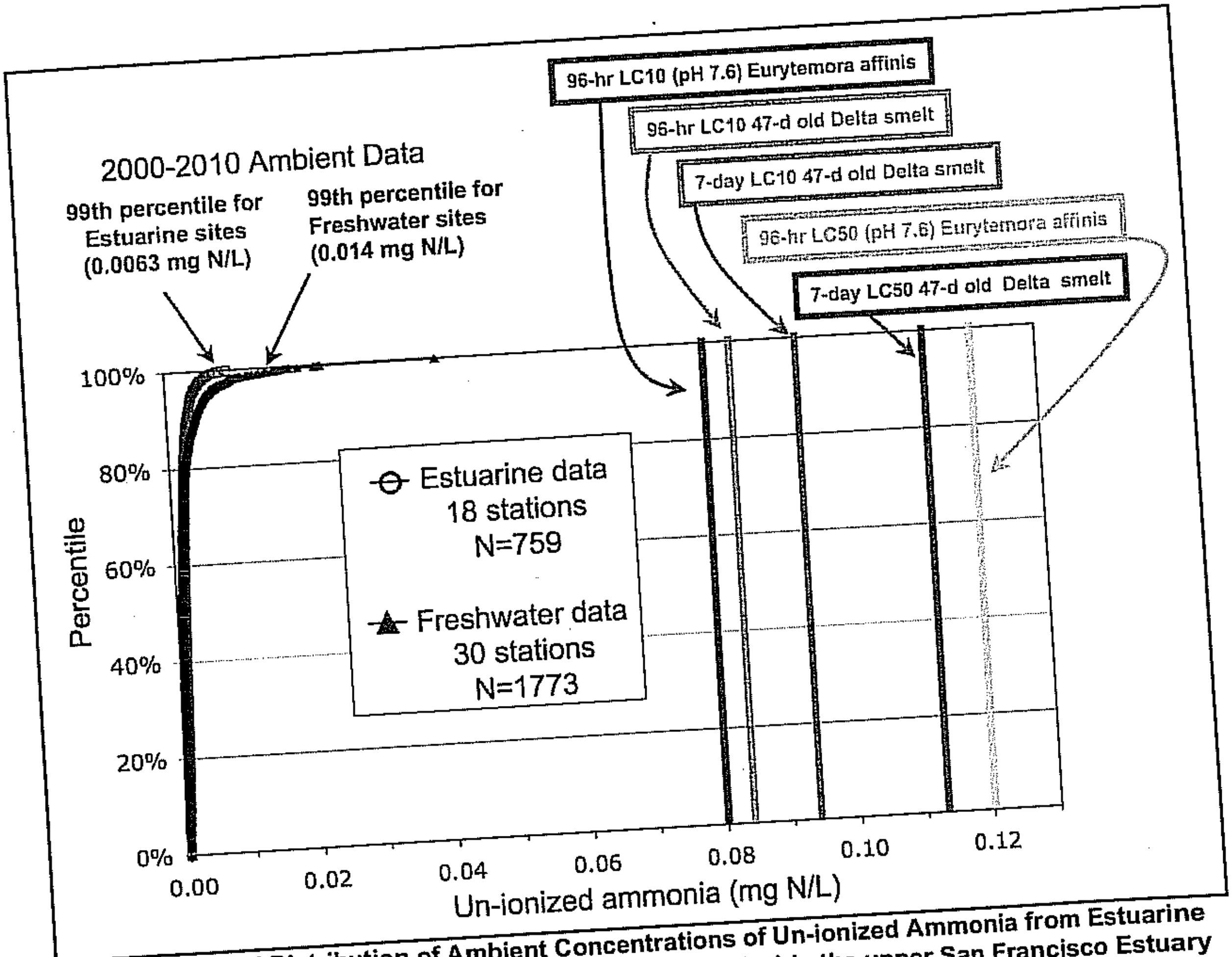

Figure 3. Ranked Distribution of Ambient Concentrations of Un-ionized Ammonia from Estuarine Stations (red circles) and Freshwater Stations (blue triangles) in the upper San Francisco Estuary for POD years 2000-2010. Datasets are described in Table 1. Included are acute effects thresholds for un-ionized ammonia from exposure tests using Delta smelt and *Eurytemora affinis*. Additional effects thresholds for these species that were too high to display in the graph are provided in Table 3.

5. Use of Acute-Chronic Ratios to Infer Chronic Toxicity in the Delta

As stated in the introduction, acute-to-chronic ratios (ACRs) are being used by several investigators, in lieu of chronic toxicity test results, to postulate that ambient concentrations of ammonia in the Delta may be causing chronic toxicity to sensitive Delta species such as Delta smelt or calanoid copepods. For example, hypothetical ACRs for rainbow trout were used at the Regional Board Ammonia Summit (Werner 2009), and in recent reports to the Regional Board (Werner et al. 2009a,b), to support an argument that ambient levels of ammonia in some Delta locations may be causing chronic toxicity for Delta smelt. The logic behind the argument can be summarized as follows:

- Chronic toxicity test results are lacking for Delta smelt.
- Delta smelt appear to be as acutely sensitive to ammonia as rainbow trout (*Oncorhynchus mykiss*).
- Therefore, chronic toxicity values for Delta smelt are probably similar to those for rainbow trout.
- Hypothetical ACRs for rainbow trout are alleged to be in the range 14.6-23.5.
- One can divide the LC50 for Delta smelt (acute value) by the hypothetical ACRs for rainbow trout to estimate the concentration of ammonia that would cause chronic toxicity to Delta smelt
- Some ambient ammonia concentrations in the Delta are higher than the values that result from this exercise.

The hypothetical ACRs for rainbow trout listed above (14.6 and 23.5) are not based on evidence for chronic effects of ammonia effects on survival, reproduction, or growth of rainbow trout and were derived using test data that was excluded by USEPA in 1999 (and in the Draft 2009 update) for use in developing the chronic ammonia criterion. In fact, to date, the USEPA has determined that the available chronic test results for rainbow trout do not meet USEPA standards for use in calculating species mean chronic values (SMCVs), or for calculating a genus mean chronic value (GMCV) for its genus *Oncorhynchus*:

> "As noted in the 1999 AWQC document, five other studies have reported results of chronic tests conducted with ammonia and other salmonids including *Oncorhynchus mykiss* and *Oncorhynchus nerka*. There is a lack of consistency among the chronic values obtained from these tests, and several tests produced "greater than" and "less than" values (Table 5). Consequently, in keeping with the decision made in the 1999 AWQC document, a GMCV is not derived for *Oncorhynchus*." (2009 Draft Update of Freshwater Ammonia Criteria; USEPA 2009, p. 21)

Attachment 3 describes the derivation of the hypothetical ACRs for rainbow trout listed above, and explains why the derivation represents a significant departure from USEPA guidance concerning chronic test design and endpoints, methods for ACR derivation, and interpretation of chronic test data for the species. USEPA-vetted genus mean ACRs (GMACRs) for fish occupy the range 2.7-10.9 (USEPA 1999, 2009). In summary, assertions about chronic toxicity for Delta smelt - or other sensitive species - based on hypothetical ACRs for rainbow trout in the range 14.6-23.5 should be avoided. At a minimum, such assertions must be carefully qualified as not being based on evidence for population-level effects of ammonia on sensitive fish.

6. Ammonium Inhibition

Published observations from field surveys and microcosm experiments have indicated spring blooms of phytoplankton in Central, San Pablo, and Suisun Bays (which are dominated by large diatoms) may occur when *at least* two conditions are satisfied: (1) vertical salinity stratification improves light conditions, and (2) ambient concentrations of ammonium are below a threshold of about 4 μM (Wilkerson et al. 2006). Tracer additions during container incubations indicated that significant increases in phytoplankton biomass in water from these locations did not occur until ammonium dropped below about 1 μM, and phytoplankton uptake of inorganic N switched from ammonium to nitrate (Dugdale et al. 2007). Owing to these studies in the brackish estuary, "ammonium inhibition" of nitrate uptake, and associated delays in bloom development, have been added to the list of factors that may be affecting the base of the pelagic food web in the SFE, and are currently being investigated in the freshwater Delta.

During 2009, research addressing the relationship between ammonium, nitrate, and phytoplankton growth rates focused on the Sacramento River. Preliminary data from several synoptic surveys conducted in fall 2008 and spring 2009 by Regional Board staff, and by researchers from San Francisco State University, provide snap shots of longitudinal patterns in the Sacramento River in nutrient concentrations, phytoplankton abundance (based on pigment and particle concentrations), phytoplankton taxonomic composition (based on pigment type and size spectra of particles), primary production (based on carbon uptake rates), and rates of ammonium and nitrate uptake (based on incubations with isotopic tracers).

Some of the results of the 2008/2009 research were presented at the Regional Board Ammonia Summit (Foe 2009, Parker et al. 2009a) and in a poster presented at the September 2009 State of the San Francisco Estuary Conference (Parker et al. 2009b). <u>Several results from these studies (bulleted below) contradict elements of the ammonium inhibition hypothesis - as it applies to the freshwater Delta - and indicate that phytoplankton responses to ammonium in the Sacramento River are different than those reported from the Suisun, San Pablo, and Central Bays.</u>

- When removed from light limitation, phytoplankton accumulation was not slower in Sacramento water collected below the SRWTP discharge, compared to water collected above the discharge (see slides 8, 11 in Parker et al. 2009a).
- In the Sacramento River, maximum cell-specific uptake rates for ammonium were not lower than those for nitrate (see slides 9, 10, 11 in Parker et al. 2009a).
- Small-celled phytoplankton and green algae exhibited similar longitudinal trends as large diatoms between the Yolo/Sacramento County line and Suisun/San Pablo Bays (see figures in Parker et al. 2009b [provided in Attachment 4]).
- No step-change in phytoplankton biomass or carbon fixation rates was associated with either (1) the location of the SRWTP discharge, or (2) a shift from primarily nitrate uptake by phytoplankton to primarily ammonia uptake below the discharge. Carbon fixation rates decreased upstream of the SRWTP, despite the fact that nitrate dominated N uptake in that reach of the river (see figures in Parker et al. 2009b [provided in Attachment 4]).
- Significant increases in phytoplankton concentration and carbon fixation can occur between Rio Vista and Suisun Bay, even when inorganic nitrogen uptake is dominated by ammonium (see slide 8 in Foe 2009b, and figure in Parker et al. 2009b [provided in Attachment 4]).
- Factors unrelated to the SRWTP discharge are apparently responsible for declines in chlorophyll-a (and other indices of phytoplankton biomass) which were observed between the Yolo/Sacramento County line and the Rio Vista locale during Spring 2009 (see slide 8 in Foe 2009b, and figure in Parker et al. 2009b [provided in Attachment 4]).

<u>The possibility is raised by these studies that ammonia inhibition (of nitrate uptake) does not influence the timing or magnitude of phytoplankton blooms in the Sacramento River (and potentially elsewhere in the freshwater Delta</u>). This possibility is supported by long term grab

sample data for chlorophyll-a and ammonia in the freshwater reaches of Sacramento River downstream of Hood (i.e., outside of the area directly or indirectly influenced by grazing by the invasive clam *Corbula amurensis*). In Figure 4, a scatter plot is presented showing available paired measurements (from monthly or bi-weekly grab samples) of chlorophyll-a and ammonium (or total ammonia) from USGS and IEP monitoring stations located between Hood and Three-Mile Slough for the period 1975-2008. Visual inspection of the scatter plot suggests that historically, high biomass of riverine phytoplankton has not been constrained to windows when ammonium concentrations were below 4 μM (equivalent to 0.56 mg N/L on the x-axis; see shaded portion of the graph). Interpretation of this type of data is limited by the frequency of collection -- ideally chlorophyll-a would be sampled more frequently to better coincide with algal blooms of short duration.

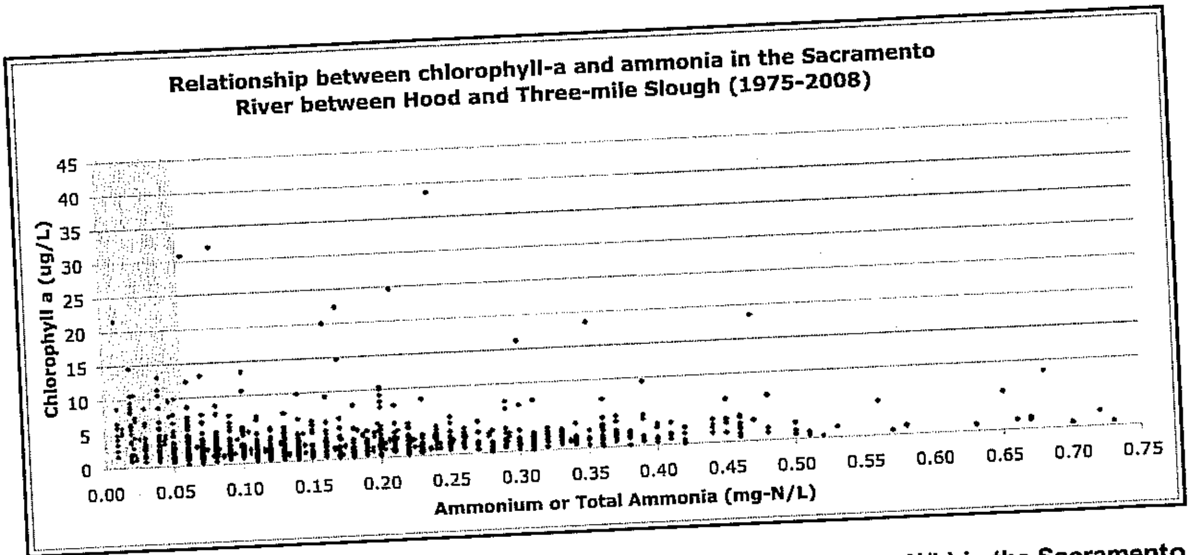

Figure 4. Relationship between chlorophyll-a (μg/L) and ammonium (mg-N/L) in the Sacramento River between Hood and Three-mile Slough between 1975-2008. Shaded area shows ammonia levels below R. Dugdale's hypothesized threshold for ammonium inhibition (4 μM, or 0.056 mg-N/L). Data are from surface water samples at USGS and IEP/DWR monitoring stations for which chlorophyll-a and either ammonium or total ammonia were measured. When ammonium data were not available, total ammonia values were used.

7. References Cited

Note: References are provided as pdfs on the CD-ROM which accompanies written testimony submitted to the SWRCB by Sacramento Regional County Sanitation District.

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Attachment 1. Inventory of Co-Occurring Measurements of pH, water temperature, and Total Ammonia from the upper San Francisco Estuary, 1974-2010

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Agency	Station Code	Station Name	Latitude	Longitude	Number of Samples		First Date	Last Date
					1974-2010	2000-2010		
Freshwater Reaches								
USGS	11303500	San Joaquin R nr Vernalis CA	37.6760406	-121.2663293	526	163	12/14/1977	1/5/2010
USGS	11447650	Sacramento R @ Freeport CA	38.45601954	-121.5013437	350	126	9/14/1977	12/16/2009
USGS	11447810	Sacramento R a Greens Landing CA	38.345745	-121.5460657	96	0	10/3/1974	9/15/1980
USGS	381427121404901	Lower Yolo Bypass nr Rio Vista CA	38.2407475	-121.6813446	2	2	3/3/2000	3/22/2000
UCD-ATL	711	Sacramento.River at the tip of Grand Island.	38.178806	-121.665306	90	90	1/12/2006	5/20/2009
UCD-ATL	812	San Joaquin River, just west of Oulton Point.	38.090306	-121.6405	50	50	2/7/2006	12/12/2007
UCD-ATL	815	San Joaquin, Confluence of Potato Slough.	38.08375	-121.572639	36	36	1/2/2008	5/20/2009
UCD-ATL	902	Old River at mouth of Holland Cut.	38.019195	-121.582194	90	90	1/12/2006	5/20/2009
UCD-ATL	910	San Joaquin River, between Hog and Turner Cut.	38.001806	-121.448694	51	51	1/2/2008	1/2/2008
UCD-ATL	915	Old River-Western arm at railroad bridge.	37.9425	-121.5635	89	89	1/12/2006	5/20/2009
UCD-ATL	CL	Confluence of Lindsey Slough/Cache Slough	38.244222	-121.68875	38	38	1/2/2008	5/14/2009
UCD-ATL	CU	Upper Cache Slough – mouth of Ulatis Creek	38.284083	-121.717861	38	38	6/7/2007	5/20/2009
UCD-ATL	Hood	DWR Water Quality Monitoring Station	38.367667	-121.520444	53	53	5/8/2007	5/12/2009
UCD-ATL	Light 55	Sacramento River Deep Water Channel at Light 55	38.274028	-121.661917	89	89	2/7/2006	5/14/2009
UCD-ATL	R&R	San Joaquin, Rough & Ready Island	37.957908	-121.357763	39	39	1/4/2008	5/12/2009
SRCSD CMP	Freeport	Freeport	38.4855	-121.5091667	44	44	1/11/2001	1/4/2008
SRCSD CMP	River Mile 44	River Mile 44	38.43467	-121.51917	45	45	7/10/2002	6/12/2008
IEP-EMP	C10	San Joaquin River near Vernalis	37.67575	-121.265	311	0	1/21/1975	12/11/1995
IEP-EMP	C10A	San Joaquin River near Vernalis	37.6793	-121.2651	11	11	3/18/2009	1/5/2010
IEP-EMP	C3	Sacramento River @ Greenes Landing	38.34575	-121.5461	332	0	1/22/1975	12/13/1995

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Agency	Station Code	Station Name	Latitude	Longitude	Number of Samples		First Date	Last Date
					1974-2010	2000-2010		
IEP-EMP	C3A	Sacramento River @ Hood	38.382	-121.519	11	11	3/18/2009	1/5/2010
IEP-EMP	C7	San Joaquin River @ Mossdale Bridge	37.786	-121.306	307	0	1/21/1975	12/13/1995
IEP-EMP	C9	West Canal @ Clifton Court Intake	37.8298	-121.5574	295	0	1/22/1975	12/11/1995
IEP-EMP	D16	San Joaquin River @ Twitchell Island	38.0969	-121.6691	328	0	1/7/1975	12/15/1995
IEP-EMP	D19	Frank's Tract near Russo's Landing	38.04376	-121.6148	327	10	1/7/1975	1/7/2010
IEP-EMP	D24	Sacramento River below Rio Vista Bridge	38.15	-121.7	340	0	1/7/1975	12/19/1995
IEP-EMP	D26	San Joaquin River @ Potato Point	38.07664	-121.5669	355	11	1/7/1975	1/6/2010
IEP-EMP	D28A	Old River @ Rancho Del Rio	37.97048	-121.573	312	11	2/3/1975	1/7/2010
IEP-EMP	MD10	Disappointment Slough @ Bishop Cut	38.04381	-121.4188	295	0	1/21/1975	12/8/1994
IEP-EMP	MD10A	South channel of Disappointment Slough	38.04381	-121.4188	22	10	1/23/1995	1/6/2010
IEP-EMP	MD6	Sycamore Slough near Mouth	38.1415	-121.4687	98	0	2/4/1975	9/27/1983
IEP-EMP	MD7	South Fork Mokelumne below Sycamore Slough	38.12513	-121.497	122	0	2/4/1975	9/27/1983
IEP-EMP	MD7A	Little Potato Slough @ Terminous	38.11382	-121.498	180	0	1/10/1985	12/14/1995
IEP-EMP	P10	Middle River @ Victoria Canal	37.8912	-121.4894	102	0	3/22/1976	9/20/1982
IEP-EMP	P10A	Middle River @ Union Pt.	37.89126	-121.4883	191	0	10/5/1982	12/13/1995
IEP-EMP	P12	Old River @Tracy Road Br.	37.805	-121.449	247	0	1/22/1975	8/16/1991
IEP-EMP	P12A	Old River @ Oak Island	37.80284	-121.4569	63	0	9/4/1991	12/11/1995
IEP-EMP	P2	Mokelumne River @ Franklin Road bridge	38.25542	-121.4403	29	0	1/21/1975	12/15/1977
IEP-EMP	P8	San Joaquin River @ Stockton aka Buckley Cove	37.97817	-121.3823	332	10	2/3/1975	1/6/2010
DWR-MWQI	B0702000	San Joaquin R. nr. Vernalis	37.67611111	-121.2641667	194	136	7/18/1996	12/15/2009
DWR-MWQI	B9591000	Contra Costa PP Number 01	37.97888889	-121.7008333	92	71	6/6/1996	2/3/2009
DWR-MWQI	B9C74901336	DMC Intake @ Lindemann Rd.	37.81611111	-121.56	16	0	6/13/1996	9/2/1997
DWR-MWQI	B9D43434343	Barker Slough @ Cook Road	38.28416667	-121.8227778	1	0	1/29/1998	1/29/1998
DWR-MWQI	B9D74711184	San Joaquin R. @ Mossdale Bridge	37.78611111	-121.3058333	15	0	6/13/1996	9/2/1997
DWR-MWQI	B9D75351293	Middle R. @ Borden Hwy.	37.89111111	-121.4888889	15	0	6/12/1996	9/3/1997
DWR-MWQI	B9D75351342	Old R. nr. Byron (St 9) (near Hwy 4 Bridge)	37.89111111	-121.5691667	105	84	6/12/1996	2/3/2009

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					1974-2010	2000-2010		
DWR-MWQI	B9D75811344	Old River at Bacon Island	37.96944444	-121.5711111	113	85	6/12/1996	2/3/2009
DWR-MWQI	B9D81561483	Calhoun Cut at Hwy 113	38.26027778	-121.8044444	14	0	1/29/1998	6/24/1998
DWR-MWQI	B9D81651476	Barker Slough Near Pumping Plant	38.27472222	-121.7933333	12	0	3/25/1998	6/24/1998
DWR-MWQI	B9D82071327	Sacramento River at Greene's Ldg.	38.34583333	-121.545	21	0	6/5/1996	5/4/1998
DWR-MWQI	B9D82211312	Sacramento R @ Hood	38.36861111	-121.5205556	134	131	6/10/1998	1/4/2010
DWR-MWQI	KA000000	Clifton Court Intake	37.82978056	-121.5573528	34	34	3/19/2007	12/14/2009
DWR-MWQI	KA000331	Delta P.P. Headworks at H.O. Banks PP	37.80194444	-121.6202778	151	115	6/13/1996	12/16/2009
		Estuarine Reaches						
UCD-ATL	323	San Pablo Bay, Rodeo Flats opposite end of rock wall.	38.048306	-122.282806	14	14	1/25/2006	7/27/2006
UCD-ATL	340	Napa River along Vallejo seawall and park.	38.0975	-122.262194	57	57	1/25/2006	5/13/2009
UCD-ATL	405	Carquinez Straight, just west of Benicia army dock.	38.039694	-122.1505	70	70	1/25/2006	5/18/2009
UCD-ATL	504	Suisun Bay, east of middle point.	38.0545	-121.9895	51	51	1/12/2006	12/13/2007
UCD-ATL	508	Suisun Bay, off Chipps Island, opposite Sacramento North ferry slip.	38.0455	-121.918806	87	87	1/12/2006	5/18/2009
UCD-ATL	602	Grizzly Bay, northeast of Suisun Slough at Dolphin.	38.114	-122.046194	89	89	1/25/2006	5/18/2009
UCD-ATL	609	Montezuma Slough at Nurse Slough.	38.167194	-121.938	89	89	1/12/2006	5/18/2009
UCD-ATL	704	Sacramento River, north side across from Sherman Lake.	38.069167	-121.775278	50	50	1/12/2006	12/13/2007
UCD-ATL	804	Middle of Broad Slough, west end.	38.018194	-121.797	53	53	1/12/2006	12/13/2007
UCD-ATL	Napa	Napa River in Napa City @ end of River Park Blvd.	38.277694	-122.282472	28	28	1/1/2008	5/12/2009
UCD-ATL	Suisun Public Dock	Suisun Public Dock	38.236188	-122.037662	2	2	1/1/2008	1/15/2008
UCD-ATL	Suisun Slough	Suisun Slough at Rush Ranch, downstream of Boynton Slough	38.207717	-122.033048	35	35	4/15/2009	5/12/2009
IEP-EMP	D10	Sacramento River @ Chipps Island	38.04631	-121.9183	309	0	1/8/1975	12/18/1995
IEP-EMP	D11	Sherman Lake near Antioch	38.04229	-121.7995	300	0	1/7/1975	12/18/1995
IEP-EMP	D12	San Joaquin River @ Antioch Ship Channel	38.02161	-121.8063	310	0	1/8/1975	12/18/1995
IEP-EMP	D14A	Big Break near Oakley	38.01776	-121.7114	271	0	1/8/1975	12/15/1995

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Agency	Station Code	Station Name	Latitude	Longitude	Number of Samples		First Date	Last Date
					1974-2010	2000-2010		
IEP-EMP	D15	San Joaquin River @ Jersey Point	38.053	-121.688	295	0	1/7/1975	12/15/1995
IEP-EMP	D2	Suisun Bay near Preston Point	38.06544	-122.0545	13	0	1/8/1975	12/16/1975
IEP-EMP	D22	Sacramento River @ Emmaton	38.083	-121.739	335	0	1/7/1975	12/19/1995
IEP-EMP	D4	Sacramento River above Point Sacramento	38.06248	-121.8205	343	9	1/7/1975	12/10/2009
IEP-EMP	D41	San Pablo Bay near Pinole Point	38.03022	-122.3729	241	10	2/14/1980	12/14/2009
IEP-EMP	D42	San Pablo Bay near Mare Island	38.05872	-122.2847	44	0	4/8/1976	12/12/1979
IEP-EMP	D6	Suisun Bay @ Bulls Head nr. Martinez	38.04436	-122.1177	324	10	1/8/1975	12/14/2009
IEP-EMP	D7	Grizzly Bay @ Dolphin nr. Suisun Slough	38.11714	-122.0397	331	10	1/8/1975	12/11/2009
IEP-EMP	D8	Suisun Bay off Middle Point nr. Nichols	38.05992	-121.99	315	10	1/8/1975	1/8/2010
IEP-EMP	D9	Honker Bay near Wheeler Point	38.07244	-121.9392	324	0	1/8/1975	12/18/1995
IEP-EMP	S42	Suisun Slough 300' south of Volanti Slough	38.181	-122.046	85	0	2/22/1978	8/3/1984
DWR-MWQI	E0B80261551	Sacramento River @ Mallard Island	38.04361111	-121.9186111	109	85	6/6/1996	1/5/2010

Attachment 2. Formulas Used to Derive Un-ionized Ammonia Fractions and USEPA Ammonia Criteria

Calculating Salinity (ppt) from Electrical Conductivity

$$S = S_{PSS} - \frac{0.0080}{1 + 1.5 \times X + X^2} - \frac{0.0005 \times f(T)}{1 + Y^{0.5} + Y^{1.5}}$$

where,

S = salinity (ppt) (using extension of Practical Salinity Scale to low salinities [0-40])

S_{PSS} = Salinity, using Practical Salinity Scale

$$S_{PSS} = 0.0080 - 0.1692 \times R^{0.5} + 25.3851 \times R + 14.0941 \times R^{1.5} - 7.0261 \times R^2 + 2.7081 \times R^{2.5} + \Delta S$$

$$\Delta S = \left[\frac{T - 15}{1 + 0.0162(T - 15)}\right] \times \left(0.0005 - 0.0056 \times R^{0.5} - 0.0066 \times R - 0.0375 \times R^{1.5} + 0.0636 \times R^2 - 0.0144 \times R^{2.5}\right)$$

$$f(T) = \frac{T - 15}{1 + 0.0162(T - 15)}$$

$X = 400 \times R$

$Y = 100 \times R$

T = temperature (°C)

$$R = \frac{EC_s}{EC_R}$$

EC_s = electrical conductivity of sample (µS/cm)

EC_R = electrical conductivity of seawater reference (58,670 µS/cm)

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ATTACHMENT 2. Formulas Used to Derive Un-Ionized Ammonia Fractions and USEPA Criteria

SALTWATER FORMULAS

Un-ionized Ammonia in Saltwater

$$f_{NH_3} = \frac{1}{1+10^{\left[pK_a + 0.0324(298-T) + \frac{(0.0415)P}{T} - pH\right]}}$$

where,

f_{NH3} = fraction of un-ionized ammonia

$I = \frac{19.9273 \times S}{1000 - 1.005109 \times S}$ (from EPA 1989, formula 5, p. 2)[1]

$pK_a = 9.245 + 0.116 \times I$

S = salinity (ppt)

T = temperature (°K)

P = pressure (assumed to be 1 atm)

Total Ammonia Saltwater Criterion Maximum Concentration (USEPA 1989, p. 27)

$C_{CMC} = \frac{0.233}{f_{NH_3}}$ (in mg/L as N)

Total Ammonia Saltwater Criterion Continuous Concentration (USEPA 1989, p. 16, 27)

$C_{CCC} = \frac{0.035}{f_{NH_3}}$ (in mg/L as N)

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FRESHWATER FORMULAS

Un-ionized Ammonia in Freshwater (USEPA 1999, p. 2)

$$f_{NH_3} = \frac{1}{1 + 10^{pK - pH}}$$

where,

$$pK = 0.09018 + \frac{2729.92}{273.2 + T}$$

T = temperature (°C)

f_{NH3} = fraction of un-ionized ammonia

Total Ammonia Freshwater Criterion Maximum Concentration when salmonid fish are present (USEPA 1999, p. 83)

$$C_{CMC} = \frac{0.275}{1 + 10^{7.204 - pH}} + \frac{39.0}{1 + 10^{pH - 7.204}} \text{ (in mg N/L)}$$

Total Ammonia Freshwater Criterion Continuous Concentration when early life stages of fish are present (USEPA 1999, p. 83)

$$C_{CCC} = \left(\frac{0.0577}{1 + 10^{7.688 - pH}} + \frac{2.487}{1 + 10^{pH - 7.688}}\right) \times MIN\left(2.85, 1.45 \times 10^{0.028(25 - T)}\right) \text{ (in mg N/L)}$$

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Attachment 3. Evaluation of ACRs Used to Infer Chronic Toxicity for Delta smelt

Acute-chronic ratios (ACRs) are being used by several investigators, in lieu of chronic toxicity test results, to postulate that ambient concentrations of ammonia in the Delta may be causing chronic toxicity to sensitive species. For example, hypothetical ACRs for rainbow trout were used in a presentation at the Central Valley Regional Water Quality Control Board (CVRWQCB) Ammonia Summit in August 2009 (Werner 2009, slide10), and in recent reports to the CVRWQCB (Werner et al. 2009a,b), to support an argument that chronic exposure to ambient levels of ammonia in the Delta may cause toxicity for Delta smelt. This logic behind the argument can be summarized as follows:

- Chronic toxicity test results are lacking for Delta smelt.
- Delta smelt appear to be as acutely sensitive to ammonia as rainbow trout (*Oncorhynchus mykiss*).
- Therefore, chronic toxicity values for Delta smelt are probably similar to those for rainbow trout.
- Hypothetical ACRs for rainbow trout are alleged to be in the range 14.6-23.5.
- Therefore, one can divide the LC50 for delta smelt (acute value) by hypothetical ACRs for rainbow trout to estimate the concentration of ammonia that would cause chronic toxicity to Delta smelt
- Some ambient ammonia concentrations in the Delta are higher than the values that result from this exercise.

Below we provide information which shows that the hypothetical ACRs for rainbow trout stated above (14.6 and 23.5) rely on information that was excluded by USEPA in 1999 and 2009 for use in developing the chronic criterion and are not based on evidence for chronic effects of ammonia effects on survival, reproduction, or growth of rainbow trout (USEPA 1999, 2009). Consequently, inferences about chronic toxicity for Delta fish species - such as Delta smelt - based on these ACRs are questionable and should be carefully qualified.

USEPA Position on Valid Chronic Endpoints and Chronic Test Design for Fish, and Interpretation of Chronic Data for Rainbow Trout

In 1999, USEPA used explicit criteria to re-evaluate the available chronic toxicity tests for fish and aquatic invertebrates (USEPA 1999). One result of this analysis was a list of acceptable chronic tests. This list appears as Table 5 ("EC20s from Acceptable Chronic Tests") on page 65 of USEPA (1999), along with Species Mean Chronic Values (SMCV) and Genus Mean Chronic Values (GMCV) where it was appropriate to calculate them. Among the criteria for inclusion in this list were (1) the test had to be a flow-through test (except that static renewal is acceptable for daphnids), (2) test conditions had to include acceptable dissolved oxygen concentrations, and (3) the endpoint(s) of the test had to be

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survival, growth, and/or reproduction.[1] Where possible, regression analysis was used to generate EC20s for many of the acceptable studies.

In order for a chronic test to be used as part of the basis for a SMCV in USEPA (1999), it had to satisfy the definitions given in the USEPA (1985a) *Guidelines for Deriving Numerical Criteria* for a "life-cycle", "partial-life-cycle", or "early-life-stage" test. These criteria *as they apply to fish* are provided in Table 1 below.

If not meeting the criteria for any of the three test categories in Table 1, USEPA guidelines allow for *potential limited* use of data from two alternative types of tests involving fish:

1. Seven-day tests of survival, reproduction, and/or hatchability, or
2. Ninety-day tests of growth

USEPA requires that such alternative tests using *growth* as an endpoint must last for at least 90 days because reductions in weight gain for fewer than 90 days can be temporary. Per the USEPA (1985a) guidelines, neither of the two alternative types of test above should be used as the basis for a discrete chronic value for a species. However, such tests can be used as evidence for an upper limit for a chronic value (in other words, determinations that the true chronic value is *likely less than* the threshold concentration observed in the test).

The list of acceptable chronic tests for fish and their associated EC20s, and SMCVs and GMCVs (standardized to pH=8 and T=25°C) that resulted from the 1999 vetting process are provided in Table 2 below. Not all of the acceptable chronic tests included in USEPA Table 5 resulted in specific EC20s, or SMCVs. When *none* of the concentrations used in an acceptable chronic test caused significant effects on survival, growth, or reproduction, the highest concentration from the test was entered in USEPA Table 5 as ">*x*" to indicate that underlying (unknown) EC20 was not equivalent to the concentration in the table for that test, but higher than the concentration by an unknown amount. Conversely, if *all* of the concentrations used in an acceptable test caused significant effects on survival, growth, or reproduction (i.e., none of the concentrations were "no-effects concentrations", or NOECs), the lowest concentration from the test was entered in the table as "<*x*" to indicate that the underlying (unknown) EC20 was not equivalent to the concentration in the table for that test, but less than the concentration by an unknown amount. "Less than" or "greater than" qualifiers were also applied to some of the SMCVs and GMCVs calculated by USEPA.

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[1] USEPA does not utilize concentrations associated with histopathologic or behavioral endpoints (e.g. swimming speed) for SMCV derivation because they have determined that there is "no justification for equating histopathological effects with effects on survival, growth, and reproduction" (USEPA 1999, p. 45). This position is more fully explained in Appendix 5 in USEPA (1999), and was maintained in the 2009 Draft Update, released on December 30, 2009 (USEPA 2009).

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Table 1. USEPA Criteria for Life-Cycle, Partial-Life-Cycle, and Early-Life-Stage Chronic Toxicity Tests for Fish.

<table>
<tr><th>Test Type</th><th>Fish Test Criteria</th><th>Data should Include</th><th>Potentially used to Derive:</th></tr>
<tr><td>Life cycle</td><td>• Tests must begin with embryos or newly hatched young <48-hrs old
• Test must continue through maturation and reproduction
• Test should not end less than 90 days after hatching of the next generation (24-hrs for non-salmonids).</td><td rowspan="2">• Survival and growth and adults and young
• Maturation of males and females
• Eggs spawned per female
• Embryo viability (salmonids)
• Hatchability</td><td rowspan="3">Depending on results:
• Upper limit for a CV
• Lower limit for a CV
• CV</td></tr>
<tr><td>Partial life cycle</td><td>• Allowed for use with fish that require more than a year to reach sexual maturity.
• Test must begin with immature juveniles at least 2 months prior to active gonad development.
• Test must continue through maturation and reproduction.
• Test should not end less than 90 days after hatching of the next generation (24-hrs for non-salmonids).</td></tr>
<tr><td>Early life-stage</td><td>• Test must begin shortly after fertilization of eggs.
• Test must continue through embryonic, larval, and early juvenile development.
• Test must continue for 60 day post hatch for salmonids (28-32 days for non-salmonids).</td><td>• Survival and growth and adults and young</td></tr>
</table>

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Table 2. EC20s and other Toxicity Parameters Accepted by USEPA (1999) from Chronic Tests Meeting USEPA Test Acceptability Criteria for Fish.

Species	EC20s	Species Mean Chronic Value at pH=8 & 25°C (mg N/L total ammonia)	Genus Mean Chronic Value (GMCV) at pH=8 & 25°C (mg N/L total ammonia)	Genus Mean Acute-Chronic Ratio (GMACR)
Pimephales promelas (fathead minnow)	1.97 2.92 5.12	3.09	3.09	10.9
Catostomus commersoni (white sucker)	>4.79	>4.79	>4.79	<8.4
Ictalurus punctatus (channel catfish)	8.38 9.33 <8.7 to <9.9	8.84	8.84	2.7
Lepomis cyanellus (green sunfish)	7.44 4.88	6.03	2.85	7.6
Lepomis macrochirus (bluegill)	1.35	1.35		
Micropterus dolomieu (smallmouth bass)	3.57 4.01 6.5 4.65	4.56	4.56	7.4
Oncorhynchus clarki (cutthroat trout)	<19.7	Not Available: USEPA determined it was inappropriate to calculate SMCVs for *Oncorhynchus* species (see text).	Not Available	Not Available
Oncorhynchus mykiss (rainbow trout)	>5.4[a] <18.7[b] <1.44[c] 1.34[d]			
Oncorhynchus nerka (sockeye salmon)	<4.16			

(a) based on the highest concentration tested by Thurston et al. (1984)
(b) based on LC50s obtained over 42-days by Burkhalter & Kaya (1977)
(c) based on 73-day LC20 obtained by Solbe & Shurben (1989)
(d) based on test results by Calamari et al. (1977, 1981), interpolated by USEPA to estimate a 72-day LC20

USEPA determined that EC20s from five tests using rainbow trout were from acceptable chronic tests. However, as a group, the EC20s for rainbow trout did not meet USEPA standards for further use in calculating SMCVs, or for use in calculating a GMCV for its genus *Oncorhynchus*:

> "Because of the concerns about some of the tests, the differences among the results, and the fact that some of the results are either "greater than" or "less than" values, and the fact that some of the results are included in Table 5, a SMCV is not derived for rainbow trout even though the various results are included in Table 5, a SMCV is not derived for rainbow trout; instead the results of the chronic tests will be used to assess the appropriateness of the CCC". (USEPA 1999; p. 60)

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No additional chronic test results for rainbow trout were included in the recently released USEPA Draft 2009 Update for the freshwater ammonia criteria (USEPA 2009), in which USEPA again declined to calculate a GMCV for *Oncorhynchus*.

> "As noted in the 1999 AWQC document, five other studies have reported results of chronic tests conducted with ammonia and other salmonids including *Oncorhynchus mykiss* and *Oncorhynchus nerka*. There is a lack of consistency among the chronic values obtained from these tests, and several tests produced "greater than" and "less than" values (Table 5). Consequently, in keeping with the decision made in the 1999 AWQC document, a GMCV is not derived for *Oncorhynchus*. Instead, the results of the chronic tests were used to assess the appropriateness of the CCC." (USEPA 2009, p. 21)

In Appendix 7 of USEPA (1999), Acute-Chronic Ratios (ACRs) were calculated for all EC20s that *were* used to generate SMCVs (from USEPA 1999, Table 5) *and* which could be paired with comparable acute values (LC50s; see more about pairing criteria below). Then, these ACRs were used to calculate Genus Mean Acute Chronic Ratios (GMACR). This analysis resulted in GMACRs for five genera of fish, which are included in Table 2. ***The USEPA-vetted GMACRs for fish occupy the range 2.7-10.9.***

Origin of Postulated ACRs for Rainbow Trout Being Used to Infer Chronic Toxity for Delta Smelt

At the August 2009 Ammonia Summit, Dr. Inge Werner provided two values as the upper and lower limits for the ACR for rainbow trout (14.6-23.5; Werner 2009a, slide 10). The derivation of these values was not a part of Werner's talk at the Ammonia Summit. The same values were presented in the annual reports for 2008 and 2009 for the UC Davis Aquatic Toxicology Lab's Delta smelt ammonia toxicity tests (Werner et al. 2009a, b) as follows (language is from 2009 report; almost identical passage occurs in 2008 report):

> "Exposure duration is an important factor influencing the toxicity of ammonia. Seven-day toxicity tests, as performed in this study, are unable to detect the potential chronic effects of ammonia/um exposure on delta smelt. Acute-to chronic ratios are one method that has traditionally been used to extrapolate between acute and chronic toxicity when procedures for chronic testing are not available. For fish, the US EPA (1999) reports mean acute-to-chronic ammonia/um ratios for warm water fish that range between 2.7 (channel catfish, *Ictalurus punctatus*) and 10.9 (fathead minnow, *P. promelas*). <u>Cold water species such as rainbow trout, with acute ammonia/um sensitivity similar to delta smelt, have a ratio between 14.6 and 23.5, respectively (US EPA, 1999; Passell et al., 2007)</u>. If these safety factors were applied to acute effect concentrations for effluent and delta smelt larvae (7-d LC50: 3.92 mg/L)[2] then the resulting threshold concentrations for total ammonia/um would be 0.27 and 0.17 mg/L for the above safety ratios of 14.6 and 23.6, respectively. These chronic effect thresholds are below long-term average concentrations in the Sacramento River below SRWTP." (Werner et al. 2009b, page 33)

The passage above can be interpreted to mean that rainbow trout ACRs of 14.6 and 23.5 were derived by USEPA or by Passell et al. (2007). However, neither of these references provide ACRs for rainbow trout. As explained above, in 1999 and 2009, USEPA refused

[2]This appears to be a mistake in Werner et al. (2009b). 3.92 mg/L was the 7-day LOEC for this test. The LC50 was 5.40 mg/L (see Werner et al. 2009b, p. 15).

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to calculate an ACR for rainbow trout - or for even for the genus *Oncorhynchus* - owing to inadequate data. Chronic toxicity tests were not a part of the original work reported in Passell et al. (2007). As clarification, Dr. Werner explained that she had calculated the ACRs for rainbow trout as follows:

> "I used the chronic values for unionized ammonia provided in Table 3 of Passell et al. (0.031 mg/L and 0.05 mg/L), and the species mean acute value from EPA 1999 (given in total ammonia/um)[3] to calculate the corresponding value for unionized ammonia (0.728 mg/L unionized ammonia), then calculated the ratio between them [which] results in 14.56 and 23.5." (I. Werner, pers. comm., Dec. 22, 2009).

Table 3 in Passell et al. (2007) is a collection of acute and chronic values for several fish species from the literature that was included for discussion purposes in the article. In the table, Thurston et al. (1984) and Burkhalter & Kaya (1977) are cited as the original sources of the 0.031 and 0.05 mg/L un-ionized ammonia-N concentrations, respectively. The original sources of the values are not critically evaluated in the article. Below, we discuss the original studies, and associated information about them in USEPA (1999). The results indicate that the chronic concentrations Dr. Werner used to compute ACRs for rainbow trout did not meet USEPA criteria for such use.

Thurston et al. (1984). Thurston et al. (1984) was a 5-year life cycle test which exposed offspring from one pair of rainbow trout, and their F1 and F2 progeny, to the following mean concentrations of un-ionized ammonia in flow-through troughs: 0.001, 0.013, 0.022, 0.044, 0.063, and 0.074 mg N/L. Regarding this study, USEPA (1999) states that "the important data for each life stage are so variable that it is not possible to discern whether there is a concentration-effect curve" (USEPA 1999; p. 58). According to the original article, there was no significant relationship between ammonia concentration and (1) mortality of all three generations, (2) growth of F1 and F2 progeny[4], or (3) egg production. Because none of the exposure levels used by Thurston et al. (1984) caused significant effects on survival, growth or reproduction, the results of this test fell under the "greater than" category of chronic test results in USEPA (1999). In other words, USEPA concluded that the underlying (unknown) chronic value for rainbow trout must be greater than the highest test concentration used in the study (5.4 mg/L total ammonia-N at pH=8, T=25°C).

Passell et al. (2007) do not explain why they identified 0.031 mg/L un-ionized ammonia-N as an appropriate chronic value from Thurston et al. (1984), or why it merited status as one of only two chronic concentrations for rainbow trout to include in their article. Because *none* of the test concentrations in Thurston et al. (1984) resulted in significant effects on survival, growth, or reproduction for 3 generations of fish, no EC20s (or other effects concentrations) are available from this test for approved endpoints. Earlier USEPA criteria documents (Table 2 in both USEPA 1985b, 1989) list 0.031 as a chronic

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[3] The species mean acute value for rainbow trout in USEPA (1999) is 11.23 mg/L total ammonia-N (standardized to pH=8, 25°C).

[4] It was not possible to evaluate growth of the parental fish because they were not weighed at the start of the test.

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value for Thurston et al. (1984) which - after comparison of the original article with associated text in USEPA 1984 - appears to have been calculated using a NOEC and LOEC related to epidermal cell changes. However, this interpretation of the results from Thurston et al. (1984), which depends on the use of a non-conventional endpoint, was rejected in both of the most recent USEPA criteria documents (1999, 2009).

Burkhalter & Kaya (1977). Burkhalter & Kaya (1977) did not report EC20s for rainbow trout. Instead, they reported LC50 results from a 42-day exposure of rainbow trout embryos and sac fry. Because the study did not provide EC20s, the results of this test fell under the "less than" category of chronic test values. In other words, USEPA concluded that the underlying (unknown) chronic value would have been less than the LC50 from their study (18.7 mg/L total ammonia-N at pH=8, T=25°C). However, the value of 0.05 mg/L unionized ammonia-N attributed to Burkhalter & Kaya (1977) in Passell et al.'s table (which was ultimately used by Dr. Werner to generate one of her ACRs for rainbow trout) is *not* that associated with the LC50 from their study (which was 0.25 mg/L unionized ammonia-N). The only available explanation for Passell et al.'s identification of 0.05 mg N/L as a chronic value from Burkhalter & Kaya is that 0.05 mg N/L was the lowest exposure concentration they used, which caused "some retardation of early growth and development" (quote from abstract of Burkhalter & Kaya). However differences in growth rate at this low test concentration (0.05) compared to the control were slight, and disappeared after two weeks of exposure. Because of the short duration of Burkhalter & Kaya's test, it was not considered by USEPA in 1999 as an appropriate test to gauge the effects of ammonia on growth on early life stages of rainbow trout.

As indicated above, Thurston et al. (1984) and Burkhalter & Kaya (1977) are discussed in USEPA (1999) and were two of the rainbow trout studies included in the list of acceptable chronic studies (see EC20 values in Table 2 above). However, as explained above, after re-evaluation of these two studies, USEPA interpreted the results of these two studies as evidence for an EC20 *greater than* 5.4 mg/L total ammonia-N (Thurston et al. study) and *less than* 18.7 mg/L total ammonia-N (Burkhalter & Kaya study; both values standardized to pH=8, 25°C). Taken in isolation from other chronic tests, USEPA's upper and lower limits from these two studies imply that the rainbow trout ACR falls somewhere within the range (0.60-2.08)[5] - which is very different than the one proposed by Dr. Werner (14.6 - 23.5).

A recent 90-day chronic test measuring the hatching success of newly fertilized eggs from a wild strain of rainbow trout, and subsequent survival and growth of sac fry and swim-up fry (Brinkman et al. 2009), resulted in a chronic value (the geometric mean of the LOEC and NOEC) of 8.06 mg/L total ammonia-N and a 90-day EC20 (based on biomass) of 5.56 mg/L total ammonia-N (standardized to pH 8). This test appears to meet the USEPA criteria for early-life-stage tests for salmonids outlined in Table 1; an ACR for rainbow trout based on the chronic value from this recent test would be about 1.4. However, even if Brinkman et al. (2009) was added to its list of acceptable chronic

[5] 11.23/18.7 = 0.60; 11.23/5.4=2.08

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tests[6], USEPA might still conclude that chronic test data for rainbow trout are too variable, or otherwise insufficient, to calculate SMCVs, or an ACR for the species or the genus.

In general, the approach of pairing acute values and chronic values from different investigations to compute ACRs is not necessarily in agreement with USEPA guidelines. USEPA (1985a), outlines the following steps for producing an ACR from a chronic value:

1. The numerator for the ACR should be the geometric mean of the acute values for that species from all acceptable flow-through acute tests in the same dilution water.
2. For fish, the acute tests should have been conducted with juveniles.
3. The acute tests should have been (a) a part of the same study as the chronic tests, (b) from different studies but from the same laboratory and dilution water, or (c) from studies at different laboratories using the same dilution water.
4. If no such acute tests are available, an ACR should not be calculated.

Conclusion

In summary, *based on the most recent USEPA criteria for chronic test design and endpoints, derivation of ACRs, and interpretation of data from chronic tests for fish*, no information is available to support a proposal that the ACR for rainbow trout occupies the range 14.6-23.5. Derivation of hypothetical ACRs for rainbow trout as high as the ones used at recent meetings and reports is not possible using direct evidence for chronic effects of ammonia on survival, growth, or reproduction and represents a significant departure from current USEPA guidance concerning the use of data from chronic tests for the species. Assertions about chronic toxicity in the Delta that rely on these hypothetical ACRs for rainbow trout should be avoided. At a minimum, such assertions must be carefully qualified as not being based on evidence for population-level effects of ammonia on sensitive fish.

[6] Brinkman et al. (2009) was published after the Feb. 2009 cut-off for the literature review used for the development of the USEPA 2009 Draft Update of the freshwater ammonia criteria.

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Note: References below were provided as pdfs on the CD-ROM which accompanied written testimony submitted to the SWRCB by Sacramento Regional County Sanitation District.

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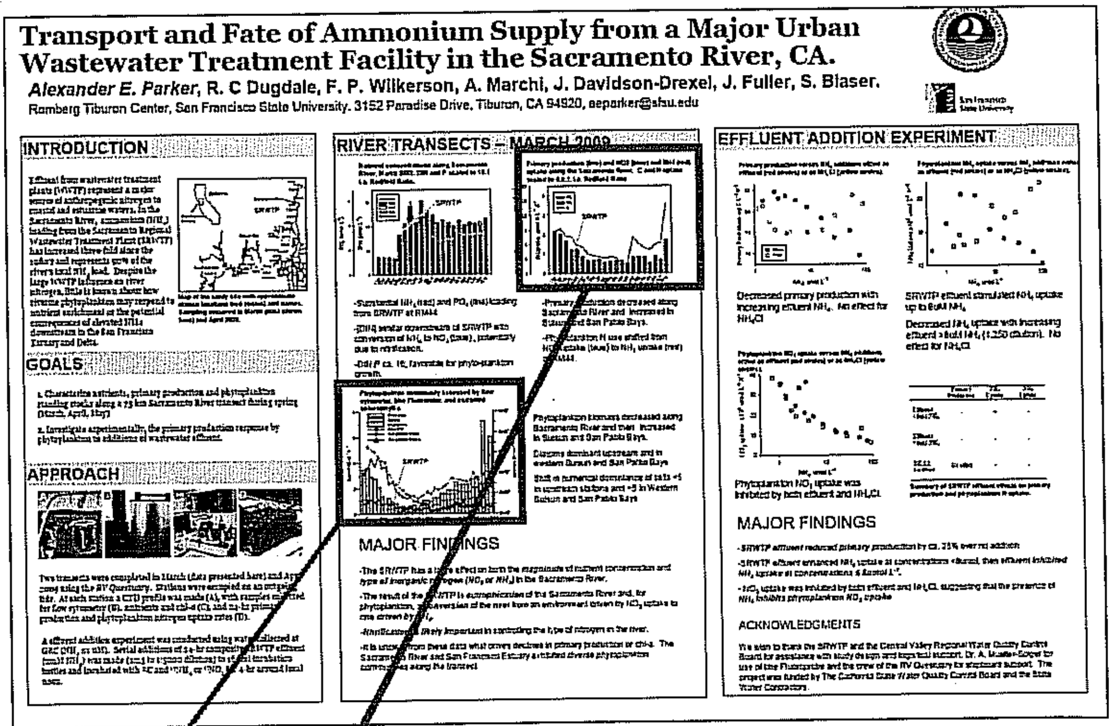
Attachment 4. Figures from Parker et al. 2009b

Note: pdf of complete poster is included on the CD Rom of SRCSD exhibits



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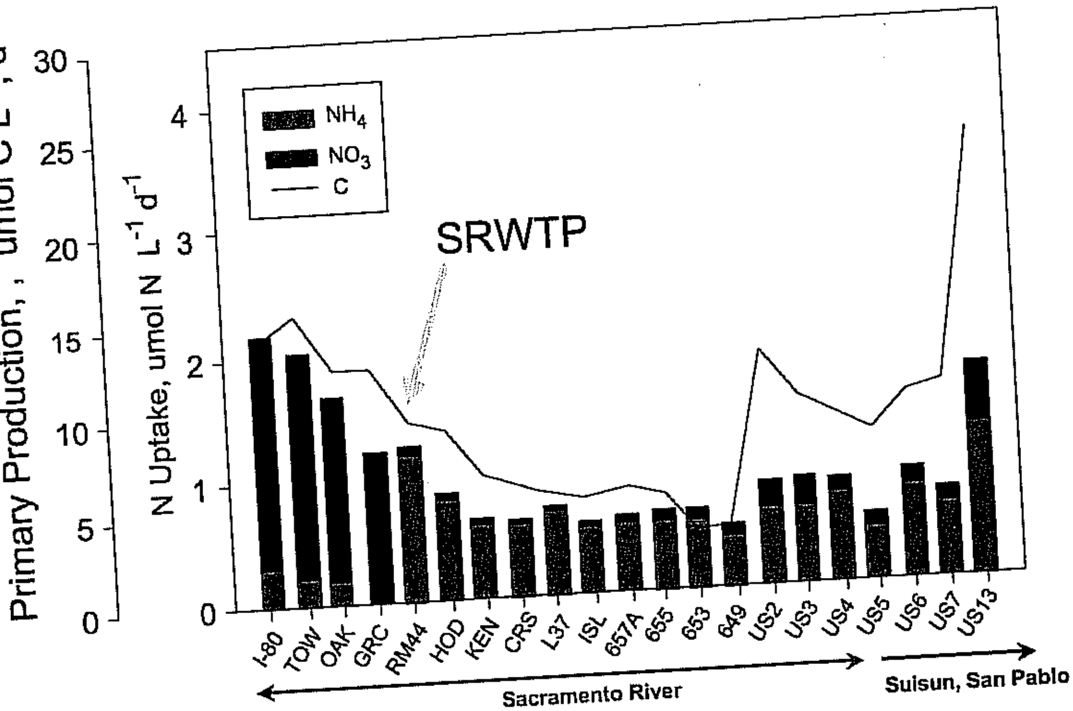

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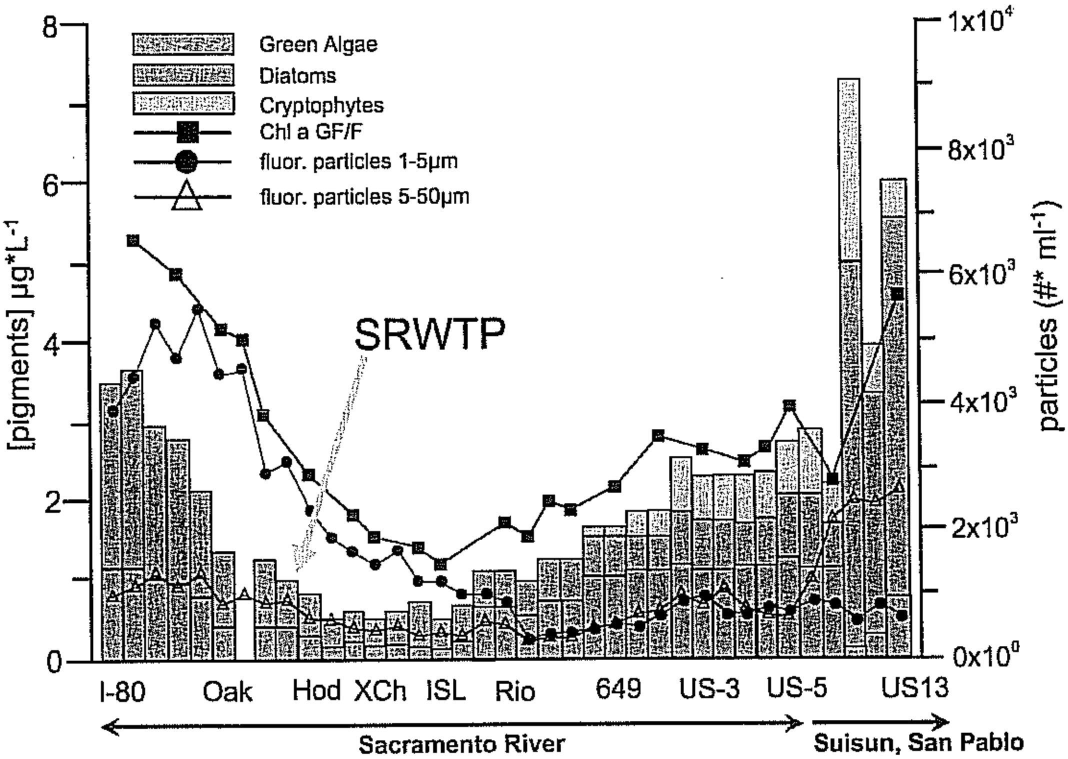

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NACWA
A Clear Commitment to America's Waters

National Association of Clean Water Agencies

Whole Effluent Toxicity (WET) NPDES Permit Testing and Limitations for Public Agencies

White Paper

January 2006

TABLE OF CONTENTS

A. Introduction and Background

EPA's National Pollutant Discharge Elimination System (NPDES) program regulations require "whole effluent toxicity" (WET) effluent testing by publicly owned wastewater treatment facilities (POTWs) during each permit cycle. The regulations also require (with certain exceptions) that NPDES permits include limitations for WET, if WET data demonstrate that the facility has "reasonable potential" (RP) to cause or contribute to a receiving water excursion above either (1) numeric state water quality standards for WET or (2) the state's narrative ("no toxics in toxics amounts") water quality standard. These requirements are of substantial concern for clean water agencies because (1) WET permit limitations often pose difficult compliance challenges, (2) it is difficult and expensive to determine and/or correct the underlying cause of WET exceedances, (3) testing is expensive, and (4) like any permit requirement, once imposed, limitations are difficult to remove.

WET testing involves the exposure of cultured test organisms to varying concentrations of effluent, measurement of biological responses (mortality, growth, reproduction), and the calculation of an endpoint or effluent concentration at which the measured effect exceeds that of the control (a test performed on dilution water without effluent) or exceeds a predetermined measure of "toxicity." EPA and state NPDES agencies generally presume that an observed difference from control organism response indicates "toxicity" and from that extrapolate receiving water toxicity or a narrative water quality standard excursion. This is what leads to either numeric permit limits for WET or other permit conditions designed to confirm, determine the cause of, and eliminate "toxicity" in the effluent.

The National Association of Clean Water Agencies (NACWA), along with several state POTW organizations and industrial groups, challenged EPA's 2002 repromulgation of WET test methods in federal court. For strategic reasons the appeal focused on the most problematic sublethal endpoints within the chronic methods. NACWA believes particularly that for those methods and endpoints, the test methods are simply not accurate or precise enough for the NPDES program uses called for by EPA and state regulations, and they do not accurately predict receiving water conditions or "toxicity."[1] This is especially so where there are relatively low levels of "toxicity," as is often the case with POTW effluents. Although the appeal was aggressively pursued, the Court of Appeals for the District of Columbia Circuit upheld the test methods in December, 2004.[2] Nonetheless, the decision supported only the methods themselves and specifically did not support any particular NPDES permitting use of WET. The Court provided valuable input on proper and improper uses of WET methods. That input, the use of which this White Paper addresses, included:

- The Court's warning against the use of single WET test failures to bring enforcement actions. EPA's permitting system must account for the fact that sometimes a test will give a correct result, and sometimes the test will report (for example) twice the "true" level of toxicity.[3]

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[1] Because NACWA believes that WET test results alone are inadequate to identify instream toxic conditions, this White Paper generally places the term "toxicity" in quotation marks.

[2] Edison Elec. Inst., NACWA, et al. v. EPA, et al., No. 96-1062 (D.C. Cir. Dec. 10, 2004) (rehearing denied 2005) (hereinafter "WET Court Decision") (Appendix A).

[3] Id. at 9.

- The validity of the methods does not imply the validity of any particular result.[4]
- Although more general Clean Water Act case law supports the near-finality of test results reported on a Discharge Monitoring Report (DMR), the Court noted that for WET methods nothing "forecloses consideration of the validity of a particular test result in an enforcement action."[5]
- States have the discretion to set toxicity thresholds to compensate for local conditions at the permitting stage. This may mitigate the lack of correlation between tests results and instream impact ("representativeness"), particularly at low levels of toxicity. EPA must establish representativeness in permitting.[6]
- Individual permits may be challenged if the clean water agency believes that regulation of toxicity is at a level posing minimal risk.[7]
- Permitting systems must account for the imprecision inherent in WET data.[8]

The development and presentation of the case by NACWA and the other petitioners also served to expose and better develop parts of EPA's rulemaking record. This provided valuable information and data on WET issues that clean water agencies will be able to use to their advantage in permit proceedings and in working with state agencies on WET programs.

Based on the WET Court Decision and the experience that NACWA members have gained in dealing with WET permitting among the states and EPA Regions, NACWA has assembled this White Paper. The White Paper includes guidance for working with NPDES permit agencies in developing (1) WET testing conditions, (2) endpoints for judging results, and (3) permit conditions that provide for (based on WET results) moving to less frequent routine testing, toxicity determination procedures, and numeric permit limits when justified. This White Paper also provides suggested NPDES permit language taken from permit language currently used by the states.

The difficulties of WET permitting are multiplied by the fact that there have been few if any court or administrative challenges brought by permittees over WET conditions or limits. Therefore, unlike some other NPDES permit issues, the definition of the correct or incorrect way to determine reasonable potential (RP) or to make other permit decisions is based directly on the (typically very general) regulations, EPA's very stringent WET permitting guidance, and the technical details that the clean water agency is able to develop. With the WET Court Decision and the remaining legal uncertainty about the methods themselves removed, this is likely to change. But, in the meantime, a clean water agency manager will need to carefully consider the effect of WET permit conditions.

As with any NPDES permit issue, success in obtaining an acceptable permit result depends on careful preparation and definition of the issues, a solid technical and regulatory approach that considers the clean water agency's specific conditions and needs, and building a full

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[4] Id.

[5] Id.

[6] Id. at 12.

[7] Id. at 13.

[8] Id. at 8.

administrative record on which the NPDES agency may ideally make the right decision, or that will serve as a viable basis for judicial review of an improper NPDES agency decision. Although this White Paper is not intended as a litigation strategy, few difficult permit issues are successfully addressed without the detailed groundwork implied by the development of a full administrative record and the unstated possibility of review of an incorrect decision.

In an ideal NPDES permit case involving proposed WET limits, EPA or the state NPDES authority should be required to (1) prove representativeness of the specific WET endpoint(s) at the level of "toxicity" identified after instream dilution (because there are no numeric EPA water quality criteria and in most states no numeric water quality standards for WET); (2) factor WET test variabilities into any RP determination; and (3) design any WET limits in a manner that does not subject the clean water agency to undue liabilities for "false positive" results. The WET Court Decision provides support for this process. Realistically, in view of EPA's and the states' current approaches to WET permitting, achieving all of these results will be difficult in the short term. However, this White Paper provides guidance for achieving these objectives.

This White Paper provides general guidance. Because each case is unique, each must be evaluated based on its own merits and a consideration of all pertinent factors, and may need to consider factors not addressed in the White Paper. It does not provide specific legal or regulatory advice as to any individual NPDES permit, and in any such case the clean water agency manager may need to obtain case-specific legal and technical advice. The White Paper does not attempt to address EPA's Great Lakes Initiative WET procedures which are incorporated into regulation, or any specific state WET permitting guidance or program.

B. WET Testing Basics

This section provides an overview of the basics and additional details of the WET program.

1. Federal Requirements

EPA regulations impose few specific WET requirements for POTW NPDES permitting. At least four test events, using either acute (measuring short term biological impacts) or chronic (longer term – at least in relation to test organism life cycle) tests, are required for permit reissuance, with each test event on at least two different test species.[9] The regulations recommend acute tests if dilution at the edge of the mixing zone is greater than 1000:1. This extreme dilution implies little risk of chronic toxicity, and therefore focuses on acute near-field toxicity. Chronic tests are recommended if dilution is less than 100:1. If dilution is between 1000:1 and 100:1, either acute or chronic tests are appropriate. Accordingly, under the federal system or a comparable state program there is no specific regulatory basis for both acute and chronic testing.

Other than RP requirements comparable to those for chemical-specific parameters, no other specific requirements are imposed. To the extent that a state program mirrors the federal program, there is no regulatory basis for WET requirements beyond those necessary for data generation prior to reapplication, at least in the absence of prior data showing effluent "toxicity."

However, many state programs have been far more creative and apply additional WET program requirements either through statute, regulation or guidance.

[9] 40 CFR 122.21(j)(5).

2. Acute/Chronic Testing

Although generally performed on the same test species, acute and chronic testing is performed pursuant to separate test protocols. Acute tests measure organism mortality based on relatively short term exposure. Because mortality is a readily distinguishable endpoint, and (at least in a relative sense) not subject to control comparison ambiguities, most clean water agency managers believe that they are not subject to substantial risk from acute tests of misidentification of effluent toxicity ("false positives" or Type I Error). Because mortality is a severe endpoint, determination and elimination of the cause of any such toxicity may be relatively straightforward in most cases, provided the toxicity is prolonged or consistent rather than an isolated incident.

By contrast, chronic testing focuses on (in addition to mortality) more subtle endpoints, typically growth and reproduction. Because of the nature of the endpoints the risk of misidentification of toxicity is substantially greater. As noted initially, the chronic methods sublethal endpoints were the focus of the WET litigation, particularly at low levels of "toxicity" typical for POTWs. They are also the focus of this White Paper.

3. Test Organisms/Species

For freshwater testing the usual test species are fathead minnows (*Pimephales promelas*, a small fish) and daphnia (*Ceriodaphnia dubia*, a small invertebrate "water flea"). Although the federally approved methods include other species, these two are used almost exclusively by EPA and state NPDES agencies for freshwater, for both acute and chronic testing. For estuarine and marine testing the methods include the mysid (*Americamysis bahia*, a small mysid shrimp), sheepshead minnow (*Cyprinodon variegatus*) and other organisms for both acute and chronic purposes.

In an unusual twist designed to allow West Coast states to use other (presumably more sensitive) acute and chronic estuarine and marine tests organisms, EPA's 2002 repromulgation of WET methods limited the estuarine and marine methods to use with Atlantic and Gulf Coast watersheds. This raises both additional difficulties and additional legal issues for West Coast estuarine and marine dischargers.

The organisms used for WET testing are laboratory cultured and subject to control tests, intended by EPA to define normal ranges of variation in their test endpoints. However, it is NACWA's view that the quality assurance/quality control (QA/QC) for the control tests is inadequate and needs to be significantly improved. The methods have minimal requirements for control performance, they do not address variability within and across laboratories, they do not have limits for intratest control variability, and there is no national standard for reference toxicant performance.

4. Test Endpoints

The WET test endpoints are typically mortality, growth and reproduction. Mortality is expressed, through metrics addressed below, as the number or percentage of organisms that die as the result of exposure to effluent. Growth and reproduction are expressed, also through metrics addressed below, as the reduction (compared to control tests) in weight gain of the organisms or reduction in the number of offspring.

Acute test results are most commonly expressed as either the "LC50" or the "NOAEC." The LC50 is the concentration of effluent within the test at which there is 50 percent mortality (half of the test organisms die). The No Observed Adverse Effect Concentration is the concentration of effluent within the test at which there is no statistically significant mortality compared to control tests.[10]

Chronic test results are most commonly expressed as either the "NOEC" or the "IC25." The No Observed Effect Concentration is the highest concentration of effluent within the test containers at which there is no statistically significant adverse effect (decrease in survival, growth or reproduction) compared to control tests. The IC25 (25 percent inhibition concentration) is the concentration of effluent within the test at which there is a 25 percent reduction in the measured effect compared to controls. Chronic results are occasionally expressed as a "LOEC." The Lowest Observed Effect Concentration is the lowest concentration of effluent at which there is an adverse effect, a less stringent endpoint than the NOEC.

5. Hypothesis Testing/Point Estimates

The metrics for expressing biological impact differ markedly from one another. "Hypothesis testing" uses a statistical test to determine (at any particular effluent test concentration) whether the response is different (less favorable) from the control. Hypothesis testing results in the use of only one test concentration from among the several effluent test concentrations used in any WET procedure to derive the test endpoint. The NOAEC, NOEC and LOEC are hypothesis testing endpoints.

Point estimates also use a statistical procedure, but use more of the WET test data to determine the point (the test effluent concentration) at which the response is equal to a specific target. Point estimate procedures are able to interpolate between responses to each tested concentration and have at least the potential of providing a more nearly "correct" result.

Because of the use of more of the test data, point estimates are a more technically rigorous approach, and the approach that poses less risk of false positives. The LC50 (acute) and the IC25 (chronic) are point estimates. The IC25 has the additional value of using the 25 percent point as a surrogate for the detection level concept used with chemical-specific pollutant identification. That is, rather than attempting to identify the point at which there is any difference from controls, the endpoint focuses on the more distinguishable 25 percent effect point. EPA also expresses a preference for the use of point estimates.

6. Test Method Flexibility

Because of the inherent difficulties in obtaining meaningful data from live test organisms, and in response to permittees' concerns, the methods themselves provide substantial flexibility to address difficulties that may lead to inaccurate results. EPA's 1996 memorandum on flexibility correctly notes that "[t]he test method manuals do not . . . strictly prescribe every aspect of

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[10] The NOAEC endpoint was never field or laboratory validated by EPA. NOAEC data were not part of EPA's Interlaboratory Variability Study *infra*. Further, the acute test protocol references the LC50 endpoint only. Accordingly, there is a good argument that the NOAEC is not part of the adopted 40 CFR 136 Table 1A methods and should not be used for NPDES purposes.

method conduct."[11] Among other points, flexibility is provided in control of pH and resulting ammonia toxicity, temperature, hardness and test dilution concentrations.

For example, because (particularly for POTW effluents) ammonia is a common toxicant and because ammonia toxicity is highly related to pH, there is flexibility for the WET procedure to control artificial ammonia toxicity brought about by pH drift within the test. Similarly because toxicity can be influenced by water hardness (again particularly for POTW effluents that are expected to contain certain naturally occurring and added metals), provisions are made for taking test solution hardness into consideration.

C. Permitting Considerations - General

There are several WET testing considerations that affect most NPDES WET permit issues, irrespective of whether testing is monitoring for reapplication, compliance testing for numeric limits, or related to the identification and elimination of indications of "toxicity." This section provides guidance on those generic issues that may be helpful in protecting the clean water agency's interests in the NPDES permit process.

1. Frequency/Retests

WET testing is expensive and that is why most NPDES permit requirements are designed around quarterly testing (one per calendar quarter) at most. However, small numbers of data points subject the clean water agency more to the variabilities of the test procedures compared to more frequent chemical-specific testing, even if we assume that variabilities are comparable between WET and chemical methods. Although NPDES permits do not disallow the generation of additional data, there is an obvious tradeoff between costs and the value of additional data when the reliability of initial data is questionable. In critical situations, clean water agency managers often commission duplicate or repeat WET tests.

In a minor concession to the concerns of NPDES permittees, EPA has consistently stated that, in general, formal enforcement is not appropriate for single WET limit exceedances.[12] The WET Court Decision specifically endorsed this safeguard, noting that "WET tests will be wrong some of the time . . . ."[13] For chemical-specific testing, notwithstanding test variability, permittees do not generally consider that lab results that include an appropriate level of quality assurance/quality control (QA/QC) will misidentify a pollutant. However, EPA's data and the materials developed in the course of the WET litigation demonstrate that properly performed WET testing will frequently misidentify "toxicity." It is also important to note that the National Environmental Laboratory Accreditation Council (NELAC) recognizes that WET data are more variable than chemical-specific data.

An important example of this effect is provided by EPA's own data generated in support of the methods. EPA's interlaboratory testing focused on relatively toxic samples, rather than samples with more dilute or minimal toxicity which might be expected from well operated

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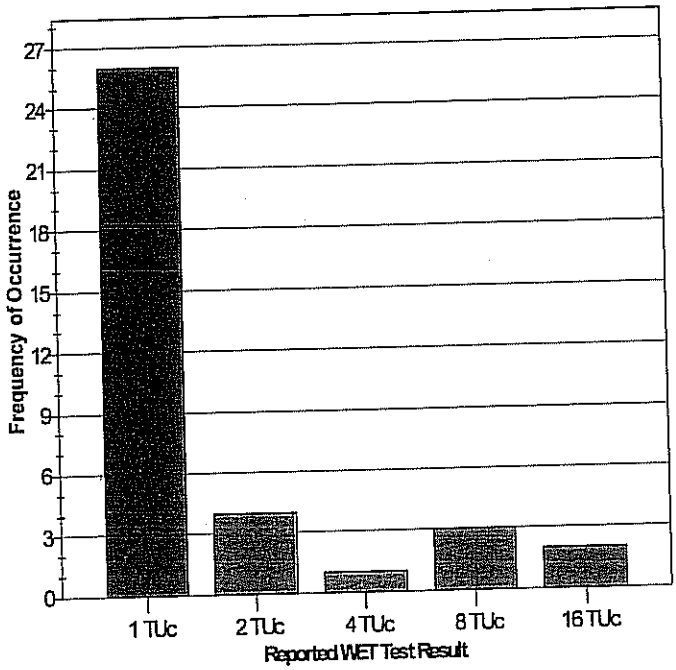
[11] Clairifications Regarding Flexibility in 40 CFR Part 136 Whole Effluent Toxicity Test Methods (EPA Apr. 10, 1996).

[12] 67 Fed. Reg. 69952, 68 (Nov. 19, 2002).

[13] WET Court Decision at 9.

POTWs. The one sample EPA tested with low toxicity had a 28 percent false positive rate[14] relative to the median response with *Ceriodaphnia* chronic results.[15] This means that (if this particular data set is representative) for a POTW with a quarterly two species test requirement (eight tests per year), on the average more than two tests per year will be expected to demonstrate "toxicity" when in fact none is present. This argues strongly for retest procedures before any finding of permit violation or before any mandatory switch to more frequent testing or TIE/TRE requirements. EPA's results are displayed graphically below.[16]

Figure 1
***Ceriodaphnia* Reproduction (NOEC) in EPA's Reference Toxicant Sample**



28% False Positive

A comparable example of variation in WET data, but on a more toxic sample, is shown by EPA's *Ceriodaphnia* reproduction data in effluent in Figure 3 *infra*. EPA's Interlaboratory Variability Study includes the comparable data for all of the repromulgated WET methods, and clean water agency managers may use those data to demonstrate the inherent variability in the methods that the agency proposes for the POTW permit. These data, and particularly graphical representations of the data, are useful in demonstrating that single WET test results should not

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14 This White Paper defines the false positive rate as the number of tests with a reported result above the central tendency of the data, divided by the total number of tests. EPA and some state NPDES agencies may use a false positive definition that does not consider all of the data above the point of central tendency to be false positives. However, using Figure 1as an example, the difference between 1 TUc (defined *infra)* and 2 TUc will typically be significant for a clean water agency, and NACWA considers that the definition used is appropriate.

15 Final Report: Interlaboratory Variability Study of EPA Short-Term Chronic and Acute Whole Effluent Toxicity Test Methods, Vol. 1 ("Interlab Variability Study"), EPA 821-B-01-004 (Sept. 2004) Table 9.8, pp. 81-82.

16 From Reply Brief of Petitioners at 26 in WET Litigation.

define NPDES permit violations and in advocating a weight-of-evidence approach to any judgements based on WET data.

2. Test Dilutions

Permit WET testing requirements frequently specify a particular "dilution series." For example, tested effluent concentrations may be 6.2, 12.5, 25, 50 and 100 percent (a "0.5" dilution series). While this may seem like a test design for identifying "toxicity" at whatever level it may occur, it is seldom an efficient test design. Rather, testing is more effectively centered on a dilution of concern (e.g., a mixing zone effluent concentration or a target concentration cited in a NPDES permit condition). That is, if a 25 percent effluent/75 percent receiving water concentration is the point at which "toxicity" or the absence of "toxicity" will be judged, test results at 100 percent are of no importance, and dilutions centered more closely on 25 percent increase the reliability of the procedures to represent potential for toxicity instream.

Although some state programs define what they consider an appropriate dilution series, these tend to be generic and ignore the site-specific nature of mixing and therefore the site-specific nature of toxicity determinations. The WET methods do not define or require specific dilution series, and the EPA's regulations do not otherwise require any particular series. Rather, test concentrations should be selected independently for each test based on the objective of the study, the expected range of toxicity, the receiving water concentration, and any available historic testing information on the effluent. Accordingly, the dilution series should be defined with regard to a particular endpoint, and both the clean water agency's and the NPDES agency's interests are served by that more specific approach. Using the 25 percent effluent example above, a 0.7 dilution series might focus on the concentration of interest and use test concentrations of 12.3, 17.5, 25, 35.7 and 51 percent. However, dilution factors much higher than 0.7 should not be used because small degrees of variance across tests dilutions may artificially result in irregular concentration-response curves or lower NOECs.

3. Data Quality Objectives

Like any laboratory program, WET testing should be approached holistically and the clean water agency manager and the laboratory should jointly determine the manner in which testing will be undertaken. Particularly when past WET results raise concerns or when the WET decision point stated in the permit or on which the NPDES agency focuses is very stringent, it is important that test problems be anticipated and guarded against. An experienced WET testing laboratory should consider and be able to specifically advise the clean water agency on method flexibility and on specific procedures that best serve to correctly identify/disprove indications of "toxicity" in a particular effluent-receiving water situation.

An assessment of concentration-response is critical. The classic toxicity response will demonstrate a consistently increasing response with increasing effluent concentration, and the laboratory should be prepared to question testing that does not identify such a response. The methods specifically provide for that level of QA/QC assessment. The methods do not specifically state that a permittee may invalidate a test purely on the basis of the concentration-response relationship. However, NACWA believes that, in the context of a full Data Quality Objectives program, the testing laboratory and the clean water agency should consider a test invalid if an adequate relationship is not present.

Data Quality Objectives must of course be implemented in a neutral manner based strictly on the merits of specific analyses. The laboratory must be prepared to disqualify data that are favorable to the clean water agency as well as data that are unfavorable. But, the laboratory should not hesitate to disqualify a WET test that does not demonstrate a proper concentration-response relationship and that otherwise does not meet proper Data Quality Objectives.

4. Expression of Results as "Toxicity Units"

EPA and generally the states express WET data not as a percent (percent of effluent in the test) having the identified biological effect as reported directly by the tests, but as "toxicity units" (TU). A TU is defined as 100 divided by the WET result expressed as percent effluent (e.g., a chronic test NOEC result of 80 percent is 100 ÷ 80, or 1.25 TUc). Among other claimed benefits, TUs are intended to avoid the counterintuitive feature of WET percent results where an increasing number indicates less "toxicity" (e.g. increasing NOEC values, up to a maximum of 100 percent, reflect increasing proportion of effluent in a test showing no observed effect).

However, the WET Court Decision raised serious concerns about the use of TUs. In response to a demonstration by NACWA and the other petitioners that WET results expressed as TUs displayed far more analytical variability than chemical-specific analytical methods, the Court concluded that the use of TUs to compute coefficients of variation (CV), an expression of variation in data from multiple tests on the same or different samples, gave a "grossly inflated result."[17]

Whether there is a substantial difference in CVs for WET data expressed as percent and as TUs is entirely dependent on the specific data set. It does appear that WET data that are generally reflective of low or nonexistent "toxicity," but with occasional outliers (a situation typical for POTWs), have the potential for the data expressed as TUs to exhibit relatively high CVs.

EPA's recommended procedures for dealing with WET data (and the procedures of many state NPDES agencies) are entirely dependent on use of TUs and the use of CVs based on TU data. However, TUs are not recognized in the WET methods themselves or in EPA regulations. Accordingly, the clean water agency manager should consider challenging the use of TUs in an appropriate circumstance. RP determinations, calculation of permit limits and any other procedures that involve the comparison and manipulation of WET data should be performed only on data expressed as percent. That approach is strongly supported by the WET Court Decision observations about the use of TUs.

The only use of WET data expressed as TUs should be as a final step after the calculation procedures, and only to take advantage of the claimed attributes of the TU metric in making increasing numeric data consistent with increasing "toxicity." The corresponding percent data should also be expressed, and any further calculations should use the percent data.

5. Data Interpretation with Hypothesis Tests

As noted above, EPA generally recommends the use of point estimates (LC50, IC25) rather than hypothesis tests (NOAEC, NOEC). However, EPA also states that both approaches produce the same result. This is generally not the case, as demonstrated by the comparisons in figures two and three and figures four and five below. To the extent that a state procedure or a NPDES permit

[17] WET Court Decision fn. 4.

requirement mandates the use of hypothesis test results, the clean water agency manager ideally should also develop and report the appropriate point estimate results.

Although QA/QC is always important when generating WET data, hypothesis test data presents an even more critical need for full QA/QC within the context of a Data Quality Objectives approach. The laboratory should pay particular attention for hypothesis tests to the PMSD limits (a QA/QC procedure specified by the WET test methods) for the chronic methods and to making sure that control test response is correct and representative. Control response is particularly important for chronic WET tests where minimum levels of performance are set by the methods (weights, fecundity, number of juveniles produced) but variability in performance within and between tests is not addressed. Although complex, control response considerations may be the difference between a WET test that exceeds a NPDES permit limit or otherwise impacts the clean water agency, and a test that can be demonstrated to atypically demonstrate "toxicity."[18]

6. Alternate Endpoint Approaches

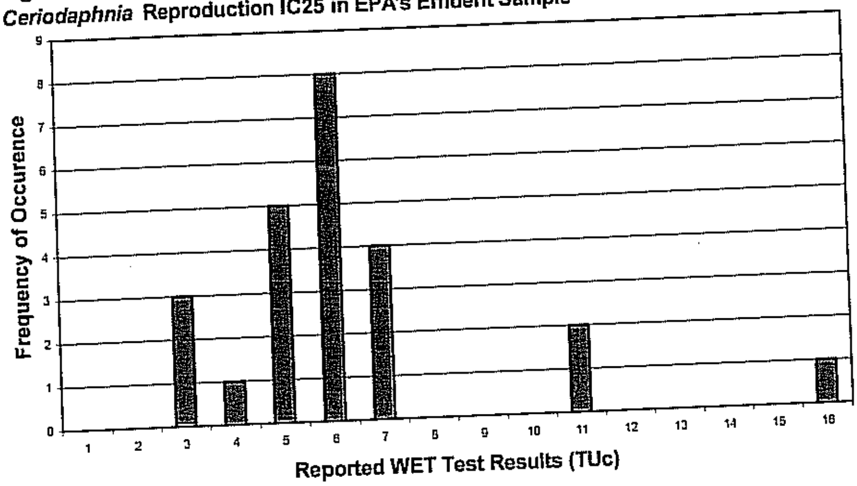
As noted above, WET acute results are typically expressed as either the LC50 or the NOAEC, and chronic results as either the IC25 or the NOEC. EPA expresses a preference for the "point estimate" LC50 and IC25, both of which make better statistical use of the data generated by the testing. The availability of different endpoints underscores the fact that there is no "correct" result in WET testing. An IC25 and a NOEC from the same test data set, both calculated correctly, will typically produce different results, and series of tests will produce different distributions of results.

This point is illustrated by considering EPA's chronic *Ceriodaphnia* reproduction data.[19] Figures 2 and 3 below, respectively, display the calculated IC25 and NOEC data from EPA's interlaboratory results on an effluent sample intended to be moderately toxic.

[18] For example, a chronic mysid test requires that the average dry weights of control organisms at the end of a test must be greater than 200 micrograms per individual. However, a testing laboratory may normally produce organisms that are 300 plus or minus 30 micrograms per individual. If a mysid test is conducted by this laboratory and the average weight of control organisms falls outside this range, a substantial case can be presented that the test is unrepresentative. Similarly, the CV for the controls may normally be 20 plus or minus 5 percent and the CV for the latest test 5 percent. Because hypothesis test endpoints are driven partially by intratest variability, this latest test may atypically predict "toxicity" where normally it would not.

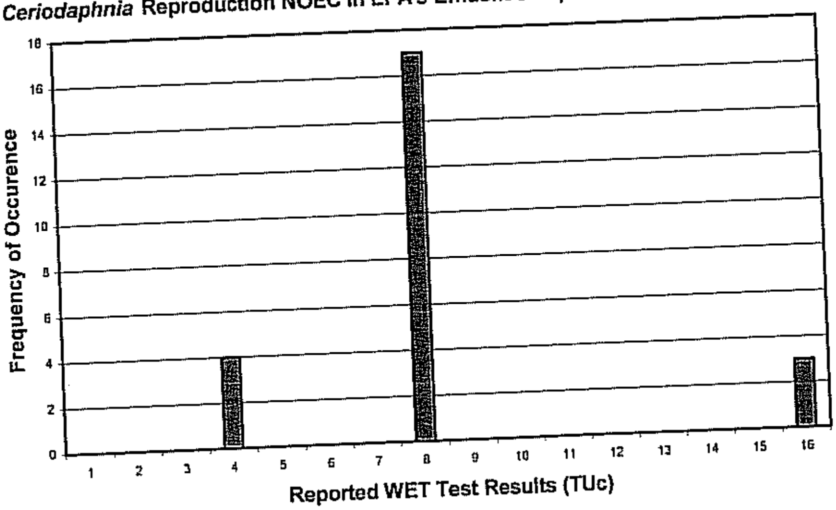
[19] Interlab Variability Study Table 9.9, pp. 83-84.

Figure 2
Ceriodaphnia Reproduction IC25 in EPA's Effluent Sample



29% False Positive

Figure 3
Ceriodaphnia Reproduction NOEC in EPA's Effluent Sample



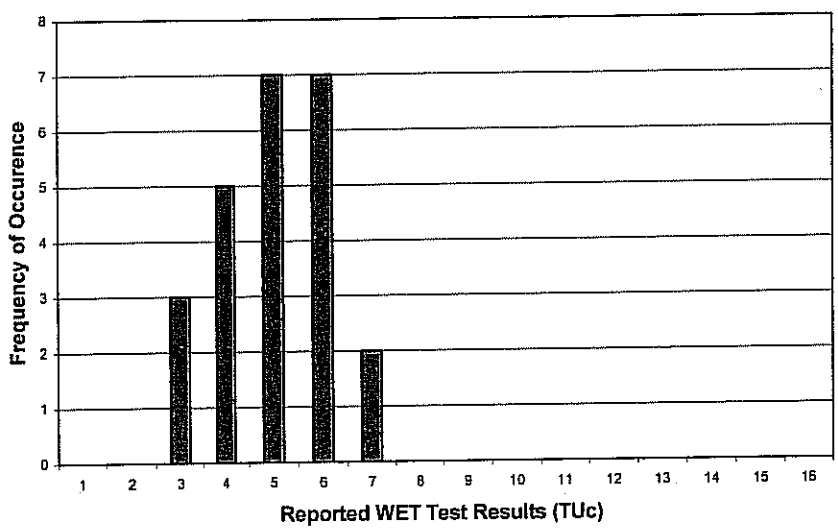
12% False Positive

First, the median, central tendency or "correct" result is different when expressed as IC25 and NOEC. In any particular permit case the distinction in the *Ceriodaphnia* example between 6 (IC25) and 8 (NOEC) Toxicity Units may be critical in determining RP or in determining compliance with a numeric permit limit. EPA's Interlaboratory Variability Study data for other test organisms and other biological effects can be readily displayed and will typically show similar comparisons. It could be observed that IC25 generally produces a "better" (less "toxicity") result. However, this is largely due to the manner in which the IC25 uses the available data, as contrasted with the NOEC identification of only the (highest effluent) concentration with no biological effect. In other words, this is largely due to a more rigorous identification of the point at which there is no "toxicity." The use of appropriate test dilutions should eliminate or moderate any such result disparity.

Second, the lesser spread of data points with the NOEC endpoint reflects the less intense use of the test data generated. Because the NOEC uses only the greatest single test concentration at which there is no adverse impact on the organisms, reported results are artificially limited to a few test concentrations. By contrast, the IC25 does an interpolation between data points, using more of the data, and will produce a more precise estimate of the effect concentration. Thru the same effect, the NOEC may generally demonstrate less data spread and a lower coefficient of variation, suggesting less analytical variability.

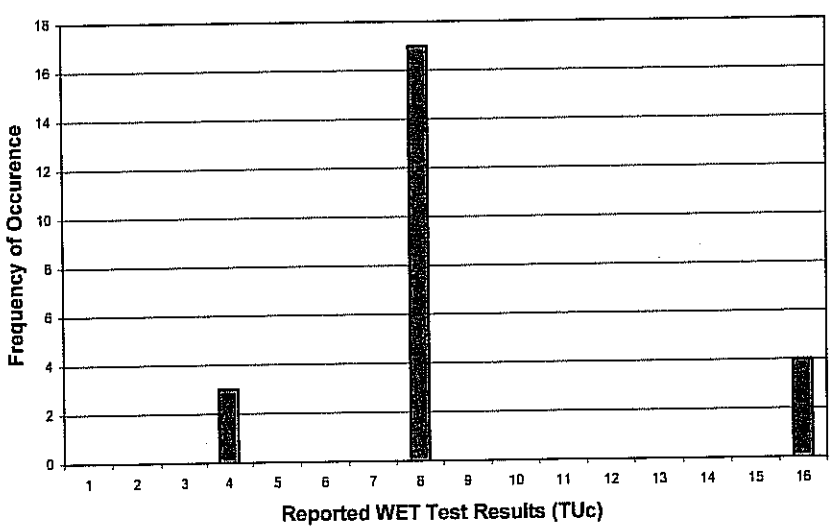
Figures 4 and 5 below illustrate similar IC25/NOEC effects for fathead minnow chronic reproduction tests.

Figure 4
Fathead Minnow Growth IC25 in EPA's Effluent Sample



8% False Positive

Figure 5
Fathead Minnow Growth NOEC in EPA's Effluent Sample



17% False Positive

Overall, NACWA believes that the endpoint comparison illustrates that (1) WET data are defined by the process from which the data are derived, rather than representing any true measure of pollutant; (2) only one endpoint and its CV and any other statistical measures should be used in any particular NPDES procedure; (3) different endpoints should not be compared, averaged or otherwise used together; and (4) irrespective of the endpoint chosen any single WET result is suspect.

In light of concerns that many clean water agency managers have expressed with use of the standard WET test endpoints, there have been calls for the development of other endpoints that could address some of these problems. In particular, some attention has focused on the Percent Effect (PE) endpoint. PE focuses testing on the instream waste concentration (IWC), and compares biological response at the IWC with a predetermined Regulatory Level (RL). The PE is the percent difference in the biological measure (survival, growth, reproduction) from the control. The procedure uses the RL as a detection level surrogate – the percent effect (difference from controls) that the WET result must exceed to be considered real toxicity. For example, a South Carolina PE approach at one point used a 40 percent RL for single WET tests.

The current use of PE or any other approach to WET endpoints is likely to raise substantial objections from EPA and perhaps from the affected state NPDES agency. The PE approach is also believed to require substantial developmental work to define a mathematical protocol for determining the PE for individual tests and for determining and justifying the RL. It is also assumed, but unproven, that PE would provide substantial benefits for NPDES permittees. However, the IC25 has some of the advantages of the PE including better use of test data and a detection level surrogate. The use of IC25, including the flexibility to focus test concentrations on the WET limit or decision point, represents the best current approach to WET permitting involving the more problematic sublethal chronic tests.

7. WET (Instream Toxicity) Criteria

Unlike most chemical-specific pollutants of interest, there are no EPA Clean Water Act section 304 water quality criteria for WET, and few if any states have adopted numeric criteria for WET within their state water quality standards. However, EPA's Technical Support Document for Water Quality-Based Toxics Control[20] (TSD) recommends a "magnitude" for WET of 1.0 TUc and 0.3 TUa, and most state NPDES agencies use those numbers. These numbers are then used as if they were instream water quality criteria, driving RP and permit limit calculations.

The chronic number is based on the belief that there should be no "toxicity" in the receiving water outside of the mixing zone.

The acute "magnitude" is based on the same idea, but because the LC50 is the point at which there is 50 percent mortality, EPA applies a theoretical 0.3 correction factor to estimate a negligible (one percent) mortality level. The basis for the 0.3 correction is questionable,[21] and a relatively small amount of laboratory work in a specific NPDES permit case may produce a

[20] EPA/505/2-90-001 (1991).

[21] EPA's TSD data show that 90 percent of the time use of the 0.3 factor overstates "toxicity."

justification for a less severe acute criterion.[22] The acute criterion is particularly onerous for many clean water agencies because, by definition, it is not a criterion that can be shown to be achieved instream, even with perfect LC50 results, unless the discharge has available dilution (for acute mix purposes) of at least 3.3:1.

Further, if acute data are expressed as the NOAEC endpoint, the 0.3 conversion has no applicability. This is because, by definition, the NOAEC identifies the effluent concentration having no mortality (unlike the LC50 which purports to identify the test concentration with 50 percent test organism mortality), and there is no correction to be made. Therefore, an acute instream number expressed as NOAEC should be no more stringent that 1.0 TUa.

In the absence of EPA or state WET criteria, state NPDES agencies should not attempt to use the 1.0 TUc and 0.3 TUa numbers as if they were binding criteria. Instead, state permit determinations of whether a POTW causes or contributes to impairment of the state's narrative criterion should be based on the totality of the evidence. That is, other data showing that the narrative criteria are protected or that instream beneficial uses are not impaired should negate any determination based solely on a calculated exceedance of the EPA numbers.

However, in response to this point EPA or the state is likely to cite EPA's policy of "independent applicability." The policy, which is not found in either the Clean Water Act or in regulations, provides that WET data, chemical-specific data and instream biological assessments should all be considered independently, and that any one (or two) types of data should not overrule an adverse indication in any other data element. As applied to the WET program, independent applicability would disallow a clean water agency demonstration based on benthic testing and aquatic life studies that, despite adverse WET test results, instream beneficial uses are not being impaired.[23 24]

In 1998 EPA issued an Advance Notice of Proposed Rulemaking in which it discussed its "current thinking" on the relative merits of independent applicability and "weight of evidence" approaches, and it specifically invited comments on alternative approaches to the use of chemical, toxicity and biological methods in determining reasonable potential.[25] No final action was ever taken on that proposed rulemaking. Further, although not disavowing independent applicability, EPA's 2006 Integrated 303(d) Report Guidance recommends that higher quality data may be weighted more favorably in water quality standards attainment determinations.[26] Although the federal NPDES program regulations arguably support the independent applicability of numeric chemical-specific standards (and the 1998 Advance Notice expressed concerns about weight of

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[22] For example, a series of acute WET tests may be designed to determine the LC50 and the LC1 on a particular effluent. The correction would be the (mean, 90th percentile or other appropriate statistic) of the ratio of the LC1 percent test result to the LC50 percent test result.

[23] See TSD Ch. 1.

[24] As to an independent applicability argument by EPA or a state NPDES agency, it is important to note that the federal regulations on WET are themselves inconsistent with a strict independent applicability approach. In addition to WET data, "other information" must inform a decision on RP, and appropriate chemical-specific NPDES permit limits may be used in place of WET limits. 40 CFR 122.44(d)(1)(v).

[25] 63 Fed. Reg. 36742, 803 (July 7, 1998).

[26] Guidance for 2006 Assessment, Listing and Reporting Requirements Pursuant to Sections 303(d), 305(b) and 314 of the Clean Water Act at IV.K (EPA July 29, 2005) (draft).

evidence primarily in that context), there is no firm legal basis for an application of the policy of independent applicability that would prevent other lines of evidence from counteracting WET data. Accordingly, clean water agency managers should not hesitate to present a demonstration that, despite an exceedance of the EPA recommended WET magnitudes, instream beneficial uses are not being impaired, and therefore the narrative water quality standard is not exceeded.

8. Instream Mixing/Dilution

Like chemical-specific pollutants, EPA and most state programs accept the concept of mixing zones within which WET numeric provisions do not apply. This is appropriate because WET effects are clearly concentration-dependent. EPA's Technical Support Document emphasizes this by noting "a discharger's chance of being charged incorrectly with causing instream toxicity is low if and only if dilution in the receiving water is considered."[27]

Because mixing will readily provide relief in terms of the stringency of WET limits, target values or other provisions, an evaluation of receiving water mixing should be an initial step in any clean water agency manager's efforts to address WET.[28] State programs frequently provide default mixing assumptions or protocols for defining acute and/or chronic mixing. Although these default procedures are typically very conservative, the clean water agency also has the option of using a "CORMIX" (a frequently used mixing protocol) or other standard or individualized mixing programs to better define mixing.

Discharges to receiving waters with tidal effects often are subject to substantially more mixing than what would be implied by steady state flow statistics. That is, the movement produced by the tides induces additional mixing. Although tidal mixing is more difficult to model than free flowing streams, modeling or empirical mixing studies are frequently worth the additional effort.

In a difficult case of application of WET limits, it may also be to the clean water agency's advantage to consider an instream diffuser in order to artificially induce additional mixing. Although this approach is seldom used for WET purposes, such use is fully consistent with frequent use of diffusers for chemical-specific purposes and with the structure of the NPDES program. Any such approach would involve design and construction costs. However, in the long term additional mixing may provide a more permanent and more reliable solution to WET results than treatment, pretreatment, incoming wastes modification or other compliance strategies. Any dilution gained also helps the clean water agency manager deal with chemical-specific water quality standards issues.

The clean water agency manager may also consider the application of Monte Carlo or other dynamic modeling procedures to assist in dilution demonstrations.[29] A Monte Carlo procedure uses a simulation involving variations in receiving stream flow, effluent volume, level of "toxicity" and any other identifiable variables affecting the occurrence of instream "toxicity." The procedure avoids the simultaneous use of multiple worst case variables, and predicts a more realistic profile of pollutant concentration or effect. Monte Carlo procedures are used with chemical-specific pollutants to examine instream impacts in conjunction with the exposure and recurrence intervals

[27] TSD Box 1-3 (emphasis in original).

[28] See TSD 2.1.1 & 5.2.2.

[29] See TSD 5.3.2.

associated with EPA's criteria and most state water quality standards. Although seldom used for WET purposes, Monte Carlo procedures have the potential to address instream impacts in a manner that is more accurate than the standard critical low flow dilution assumptions.

All of these approaches to mixing are designed to correctly reflect instream exposure to effluents. They are not devices that avoid any NPDES program requirement.

D. WET Permitting Approaches

The states' NPDES approaches to WET conditions vary from simple reissuance application requirements, to immediately effective numeric limits for WET, to complex Toxicity Identification Evaluation/Toxicity Reduction Evaluation (TIE/TRE) approaches. Each approach includes particular risks for clean water agencies. However, the WET Court Decision and the experiences of other clean water agencies provide opportunities for avoiding or improving on NPDES permit conditions that the states might otherwise impose.

1. Reapplication Monitoring Only

The only WET monitoring specifically required by the federal NPDES regulations for POTWs is for an application for permit reissuance. Reissuance monitoring should be four events, two species each event, for either acute or chronic toxicity. Further, the federal regulations do not provide a basis for a reopener provision in the event of WET data that the agency may see as justifying additional requirements. Rather, the regulations provide an exclusive list of reasons for permit modification by the agency, which do not include additional WET (or chemical-specific) data.[30] Although there is a more generic reopener provision, it is only for sludge regulation changes and other specific purposes. Accordingly, under the federal regulations, and with the possible exception of substantial new indirect dischargers or other fundamental changes inconsistent with the clean water agency's most recent permit application information, the clean water agency manager should oppose any attempt to include a permit reopener predicated on the results of WET testing. Instead, as with chemical-specific data, the five-year reapplication process is the NPDES agency's opportunity to consider RP.

Even for permits that impose only reapplication monitoring requirements, the clean water agency manager should carefully choose and work with its WET laboratory to define Data Quality Objectives and to make proper use of test method flexibility so that results reflect to the extent practical instream conditions and avoid anomalous indications of "toxicity." The clean water agency manager should work with the NPDES agency in an attempt to focus WET testing and reporting on test dilutions reflecting exposure assumptions and on the use of point estimates (LC50 or IC25).

Appendix B includes examples of appropriate reapplication monitoring requirements. As with Appendices C and D, NACWA does not consider all of the various WET provisions in the permit examples to be desirable examples for clean water agencies.

2. Routine WET Monitoring and TIE/TRE

Some state NPDES agencies impose more intensive or more routine WET monitoring, pursuant to specific state programs, because of a generic concern that treats WET data differently

[30] 40 CFR 122.62(a).

from chemical-specific data, or in response to existing POTW WET data raising concerns about "toxicity." In any such case, the clean water agency manager should address the same issues outlined immediately above for reapplication monitoring permit conditions.

As noted above, in the absence of a state regulatory provision, a permit requirement for routine WET monitoring does not have a strong regulatory basis, at least in programs similar to the federal program. However, the states often include routine monitoring in cases where existing WET data raise concerns, and it is clearly in the clean water agency's interests to have routine monitoring conditions rather than (1) numeric limits for WET or (2) a substantial debate with the NPDES agency over RP.

In any case of routine monitoring conditions, the NPDES agency obviously anticipates some use of the data if it should be unfavorable, and the clean water agency manager should work to craft appropriate WET program provisions. Those provisions would ideally include a critical WET value or target number based on averaging of multiple test results or the exceedance of critical values in at least two consecutive tests, a retest provision in the event of unfavorable results, and progression to a phased TIE/TRE program rather than numeric WET limits.

The WET Court Decision specifically supports a clean water agency's efforts in the following ways.

- The Court's observation that state NPDES agencies have the discretion to set toxicity thresholds to compensate for local conditions supports the setting of WET target numbers or thresholds that incorporate available mixing. Mixing evaluations should be undertaken when the clean water agency manager anticipates routine WET monitoring conditions because such permit conditions will typically lead to either TIE/TRE procedures or more immediate WET limits, and it will be more difficult to address mixing after the thresholds for those steps have been already established. In particular, later mixing work has missed the opportunity to focus previous test dilution series on the more correct endpoint, and has therefore missed the opportunity to maximize the reliability of the resulting data base.
- The Court's warning against the use of any single WET test result should inform the manner in which the permit handles early results before there is a substantial data set. Decisions on advancing to TIE/TRE procedures or numeric limits should only be made after there is a data set adequate to characterize the data given the variabilities in results demonstrated by EPA's interlaboratory data. EPA recommends that RP and limit determinations be based on at least ten tests.
- Building on the WET Court Decision that states that "nothing forcloses consideration of the validity of a particular test result in an enforcement action," it is also true that nothing should forclose consideration of the validity of test results applied to any permit purpose. This is where the clean water agency manager's advance work with its WET testing laboratory and the establishment of Data Quality Objectives may be critical.
- TUs should be used only as a final expression of permit target numbers. Any calculations should be performed on WET data expressed as percent.

Appendix C includes examples of appropriate routine WET monitoring provisions, though the other WET provisions are not necessarily desirable for clean water agencies.

3. Reasonable Potential Determination and Numeric WET Permit Limits

The least desirable result for a clean water agency is an NPDES permit agency determination that reasonable potential exists for the effluent to cause or contribute to an excursion beyond the state's narrative water quality standards. In any such instance the clean water agency manager's first task is to determine whether, in fact, RP exists. EPA's TSD RP procedures (which Great Lakes Initiative states and some other states use) are very restrictive, better designed for chemical-specific RP (the accuracy of chemical-specific data being determinable and CVs being more properly determinable), and would in the majority of cases without substantial instream dilution bring about a finding of RP. However, the federal regulations and many states' regulations or procedures involve a wider inquiry. The agency must consider the variability of the pollutant, the sensitivity of the WET test species, instream dilution,[31] and any other available information.[32] Instream dilution has been addressed above. In an appropriate case, the clean water agency manager should consider instream mixing evaluations or steps to modify instream dilution through a diffuser to avoid or reduce the adverse impact of WET limits. The clean water agency manager and the public should understand that both natural and induced mixing are consistent with the NPDES program, and any potential adverse effects within mixing zones are also addressed by the program. The use of mixing processes is not an avoidance of Clean Water Act responsibilities.

In terms of pollutant variability, the WET Court Decision warned of the inappropriateness of the use of single test failures for enforcement. The same principle should apply to other uses of single tests showing unusual results. In any case where there are intermittent adverse WET data, the clean water agency manager has the opportunity to evaluate its WET data in the context of EPA's interlaboratory variability data for the test species and test endpoint. EPA claims that its WET methods exhibit interlaboratory variability (CV) between 11 and 44 percent. Although NACWA believes that these CVs are systematically understated, test species with higher variabilities present a more compelling case that one or two test exceedances within a permit cycle should not be the principal basis for a RP determination. EPA's claimed CVs are provided in its rulemaking record.[33]

In terms of sensitivity of the WET test species, the WET Court Decision noted that state NPDES agencies may set "toxicity" thresholds to mitigate the lack of correlation between test results and true instream impact (representativeness). NACWA believes that representativeness, or the lack thereof, is a principal disconnect between WET testing and its use as if the data were chemical-specific data. This is particularly true for the chronic sublethal endpoints, and at relatively low levels of "toxicity" (at or near 1.0 TUc). Generally, NACWA believes that EPA does not have data that demonstrate representativeness in the absence of lethal conditions. Rather, EPA's conclusions of representativeness for the sublethal endpoints involve an assumption based on data from tests where there was lethality. Although the burden should be on the permit agency to demonstrate representativeness as to any particular RP determination, the agency is likely to rely on general EPA claims regarding its methods. In a strict legal sense such general claims, in the absence of specific data, should not be seen as supporting an RP determination. However, in a

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[31] 40 CFR 122.44(d)(1)(ii).

[32] Id. 122.44(d)((1)(v).

[33] Interlab Variability Study Tables 9.65 & 9.66, pp. 150-51.

practical sense, the clean water agency manager will need to advance the issue by raising specific doubts about representativeness.

EPA's WET rulemaking and the briefs of NACWA and the other petitioners can provide additional guidance for a demonstration concerning representativeness. For example, if a claimed RP determination were to be made using the chronic marine test methods, the clean water agency manager might take the initiative and himself cite EPA's support for representativeness. The briefing materials provide a cogent argument as to how that EPA position is without basis.[34] At this point the burden should shift back to the agency to produce specific data showing representativeness of chronic sublethal effects. NACWA found in its research that such data do not exist. EPA has stated that "no single marine case study has been designed with the goal to comprehensively evaluate [the representativeness] relationship." Also, "[w]e have discovered no case studies in the scientific literature that describe a detailed analysis of the toxicity of an effluent discharging to the estuarine or marine environments or that also describe a corresponding impact on the water column and benthic communities of the receiving system."[35] Pointedly, "WET tests originally were not designed to predict receiving system impacts."[36]

Similarly, data do not show the representativeness of the freshwater chronic sublethal endpoints. Helpful documentation is available in the WET litigation materials. However, those materials do not fully develop the freshwater issue, and additional research into EPA's Comprehensive Effluent Testing Program documents will be necessary. The CETP documents from the 1980s report on investigations of representativeness conducted on several waterways. For some of the work no WET test toxicity was identified, and the testing proved nothing.[37] For other work a relationship was purportedly identified between effluent WET test results and WET test results on receiving water samples.[38] However, this merely demonstrates that the researchers were able to identify points in the receiving waters where the appropriate concentration of effluent remained, and that the distinction between laboratory dilution water and ambient water did not make a substantial difference. In the Back River Maryland study a relationship between WET test results and instream biological quality was not identifiable.[39] Other studies purported to identify a specific relationship between WET test results on effluent and instream biological impact.[40] Such identifiable effects occurred only where there was test organism mortality, and that they prove nothing about representativeness for relatively low toxicity manifested in chronic sublethal

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[34] See, e.g. Reply Brief of Petitioners at 34 in WET Litigation.

[35] Whole Effluent Toxicity Testing: An Evaluation of Methods and Receiving Stream Impacts, Society of Environmental Toxicology and Chemistry (SETAC) section 10.3 ("Predicting Receiving Stream Impacts from Effluent Toxicity: A Marine Perspective" 1996) (the "Pellston Conference") (a "Discussion Initiation Paper" by EPA staff on behalf of EPA).

[36] Id.

[37] Validity of Effluent and Ambient Toxicity Tests for Predicting Biological Impact, Scippo Creek, Circleville, Ohio EPA 600/3-85/004 (June 1985).

[38] Validity of Effluent and Ambient Toxicity Tests for Predicting Biological Impact, Back River, Baltimore Harbor, Maryland EPA 600/8-86/001 (July 1986).

[39] Id.

[40] See, e.g. Validity of Effluent and Ambient Toxicity Tests for Predicting Biological Impact, Skeleton Creek, Enid, Oklahoma EPA 600/8-86/002 (Mar. 1986).

endpoints. However, it is this point that appears to require further research into the specific test data.

As noted above, EPA's regulations also require the consideration of any other available information as part of an RP determination. Other information may include favorable bioassessment data, favorable results of scans for chemical-specific pollutants included in the clean water agency's reapplication data, fisheries assessments from the state's environmental or fisheries resource agencies, and any other data supporting the attainment of designated uses in the receiving waters. As discussed above, EPA's policy of independent applicability should not prevent the consideration of such additional water quality data. In the absence of numeric criteria for WET within the state's water quality standards, and in the absence of a state regulation disallowing a weight of evidence approach, a demonstration that benthic metrics and water column biological integrity are not impaired (in critical low flow conditions) should offset WET data.

TUs should be used only as a final expression of permit limits or target numbers. Any calculations should be performed on WET data expressed as percent.

Appendix D includes examples of appropriate WET permit limit provisions, though the other WET provisions are not necessarily desirable for clean water agencies.

E. Additional Considerations

1. Compliance Schedules

Permit compliance schedules, whether for TIE/TRE programs or numeric WET limits should provide sufficient time for the required tasks. Under federal law and most states' programs a compliance schedule within a permit may extend at least to the end of the permit term. Given the difficulty of WET programs it would not be unreasonable in many cases to receive all or the majority of a five year permit term to complete a TIE/TRE program or to achieve compliance with numeric WET limits.

NPDES permit provisions imposing TIE/TRE requirements and eventual WET limits are best drafted to move progressively through each step, rather than providing within the permit the eventual numeric limit. For example, an appropriate compliance schedule would require workplan submission and allow a defined period to complete the TIE/TRE. Based on the program findings and conclusions, the NPDES agency would then determine whether numeric WET limits are required and, if so, what those limits will be. This avoids the argument that compliance with the limit must be within the five year permit term. It also preserves for a later time the clean water agency's arguments against WET limits or over the specific numbers. If, for example, the clean water agency's program is successful in identifying and removing an offending wastestream that had caused the "toxicity," no numeric limits should be seen as necessary. That is, with the source of the problem gone, there is no RP. Alternately, if the NPDES agency sees the problem as being addressed through new treatment processes or changes to existing processes, the agency will have a good argument that, although the "toxicity" is now gone, numeric limits are necessary to require and maintain the increased level of treatment.

2. Removing WET Limits and Permit Conditions

The removal of numeric WET limits and other permit conditions should be handled in a similar manner to chemical-specific limits and corresponding permit conditions. Numeric limits subject to a compliance schedule may be removed through permit modification prior to the date the limits become effective without violating "antibacksliding" restrictions.[41] Although EPA and state NPDES agencies will typically take the position that limits may not be removed after becoming effective, that is an overbroad reading of antibacksliding. Water quality-based limits may be revised or removed as long as (1) for waters attaining state standards, antidegradation requirements are met, and (2) for waters not yet attaining state standards, the cumulative effect of wasteload allocations provides for standards attainment.[42] Also, limits may be changed or removed if justified by alterations to the POTW, new information or other specified causes.[43] Although these provisions are complex and often poorly understood, they allow changes in or removal of permit conditions in many appropriate circumstances.

3. What Constitutes a Violation?

An exceedance of a currently effective numeric WET permit limit constitutes a permit violation and a violation of federal and state law. However, WET triggers and other conditions short of traditional limits should be carefully drafted so that the permit requires WET testing, TIE/TRE procedures and other management provisions in a way that is binding and that allows the agency to properly enforce the permit, without prematurely characterizing specific WET test results as violations. WET testing should typically be on a specified schedule and require the reporting of results with the Discharge Monitoring Report for the month of the test. A failure to test and report as required is a permit violation. Because of the difficulties inherent in WET testing, retests will frequently be required in order to obtain a test that meets the clean water agency's Data Quality Objectives and QA/QC criteria. The permit should ideally reflect this aspect of WET testing and state that, in the event of such testing difficulties, a retest as soon as practicable is in compliance with the testing requirement. Even in the absence of such a permit provision, NACWA believes that the record adequately identifies the difficulties with WET testing, and the clean water agency manager would have a viable impossibility or other defense to a charge of noncompliance based on a WET test properly rejected because of QA/QC problems.

A separate issue from retests because of Data Quality Objectives failure is retests based on WET data variability, and the fact that at least occasionally a WET test will show "toxicity" when there is no effluent toxicity.[44] NACWA believes that the record justifies a permit retest provision for any final NPDES permit numeric WET limits. However, in low dilution situations (with WET limits of 1.0 TUc or only slightly higher) a retest averaging provision is generally of little value - because no number of 1.0 TUc results will average a greater than 1.0 TUc result down to 1.0. In

[41] National Whole Effluent Toxicity (WET) Implementation Guidance Under the NPDES Program 5.3.2, EPA 832-B-04-003 (Nov. 2004) (draft) (EPA Implementation Guidance).

[42] 33 U.S.C. 1313(d)(4) & 1342 (o)(1).

[43] Id. 1342 (o)(2).

[44] WET Court Decision at 9.

recognition of this, EPA's WET Guidance approves of the use of median rather than average value in such situations, although with some substantial restrictions.[45]

TIE/TRE procedures are typically step-by-step analytical exercises where the eventual result is unknown until completion, and where the path to completion itself is unknown. Therefore, a properly constructed permit condition should require an approvable facility-specific TIE/TRE work plan by a specific date, and should require that the work plan itself include specific enforceable milestones allowing the NPDES agency to properly enforce the permit requirements. A permit violation would result if the clean water agency failed to develop the work plan on time or failed to implement and report to the NPDES agency on the specific work plan steps on time. The clean water agency manager's task is to develop the work plan in a manner that satisfies the NPDES agency's need for enforceable conditions on an identifiable timeline, which gives itself and its consultants time to perform the TIE/TRE procedures, and that where practical bases its requirements on elapsed time after the prior required NPDES agency approval rather than on a specific date.

F. TIE/TRE Requirements

1. Timeframe

NPDES permit requirements for TIE/TRE procedures should ideally not specify a date for completion. Rather, the procedure is a step-by-step process where an identification of the pollutant or condition leading to the indication of effluent "toxicity" may be identified at an initial point, or initial results may rule out a particular conclusion and require subsequent analytical steps. There is ample EPA guidance on performance of TIE/TRE procedures to establish during the permit process either an open-ended program or a program that is likely to provide enough time for completion based on past experience. Generally, NACWA believes that a permit should not anticipate TIE/TRE completion in less than two years from initiation.

2. TIE/TRE Goal

The single goal of a TIE/TRE process should be to identify the pollutant, combination of pollutants or other factors causing the WET testing indications of "toxicity," and to identify a treatment or management approach to remove or correct the cause in a manner that either (1) results in future acceptable WET test results or (2) identifies the cause of the adverse WET test results as a factor other than effluent "toxicity."

3. Degree and Variability of Toxicity

TIE procedures are only effective if sufficient toxicity is available over a number of consecutive tests. The clean water agency manager should strive for language defining these conditions if the NPDES permit agency includes language in a permit that specifically deals with this level of decision making, rather than including a permit requirement for the submission of a TIE plan that will address such details. For example, the clean water agency manager could propose that the TIE not be initiated until a pattern of significant toxicity is measured (e.g. two consecutive tests exceeding the permit decision trigger by more than 20 percent). This approach will help ensure that false positives will not drive the process. Also, there should be language addressing specifically when a TIE has been completed (e.g. two or three consecutive tests meeting

[45] EPA Implementation Guidance at 5.2.4.

the permit decision trigger). This latter language will help ensure that a TIE does not continue indefinitely.

G. Conclusions

This White Paper has presented guidance on a number of issues that may be useful for a clean water agency manager in addressing WET testing and NPDES permitting in a specific POTW permit situation. Some of the more important issues addressed in detail are listed below. Achieving an acceptable result for the clean water agency NPDES permit in a specific case may involve utilizing one or more of these issues to properly interpret and use WET data, to formulate appropriate permit conditions and convince the NPDES agency of the appropriateness such conditions, and to build an administrative record to support a correct NPDES agency decision.

- Distinctions among WET test endpoints, the metrics for expressing endpoints (e.g. the chronic NOEC and IC25), and EPA's recommendations for point estimates (e.g. IC25).
- Test method flexibility.
- QA/QC and Data Quality Objectives, and in particular concentration-response relationships.
- Frequency of WET testing, retests, and EPA and WET Court Decision statements concerning the use of single test results in enforcement.
- WET test dilutions.
- EPA data demonstrating false positive rates for the WET test methods, at least on moderately "toxic" samples.
- Use of WET data expressed as Toxicity Units, and the use of data expressed as percent (rather than TU) in any statistical or other manipulations of the data.
- Differences in WET data results between hypothesis tests and point estimates.
- Use of WET instream "toxicity" criteria.
- Instream mixing and dilution.
- Proper expression of NPDES permit requirements for reapplication and routine WET monitoring.
- Triggers for TIE/TRE requirements, and effective TIE/TRE permit conditions.
- Representativeness, or the degree to which WET data accurately predict instream toxicity.
- The use of state narrative water quality standards, RP, and NPDES permit numeric limits for WET.